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A. D. MELVIN, CHIEF OF BUREAU.

THE BACTERIOLYTIC POWER OF THE BLOOD SERUM OF HOGS.

BY

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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., December 27, 1906.

SIR: I have the honor to transmit for publication the accompanying paper entitled "The Bacteriolytic Power of the Blood Serum of Hogs," by B. M. Bolton, M. D., of the Biochemic Division of this Bureau.

The experiments herein described were carried out with certain strains of the hog-cholera bacillus, and are a part of the general investigations concerning hog cholera which have been conducted for some years by the Biochemic Division. The subject of bacteriolysis has claimed much attention from bacteriologists in recent years, and Doctor Bolton's work is believed to be of interest on account of the light it throws upon the defensive mechanism of the animal body in its fight against infectious diseases.

I recommend that the paper be published in the bulletin series of this Bureau.

Respectfully,

A. D. MELVIN,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

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THE BACTERIOLYTIC POWER OF THE BLOOD SERUM OF HOGS.

INTRODUCTION.

Aside from a few preliminary experiments with cultures of the typhoid and colon bacilli, the present investigation was confined to the reactions taking place between three strains of *B. choleræ suis*, and normal and immune serum of hogs.

The bactericidal properties of the blood serum and other fluids of the body have of recent years attracted a great deal of attention from bacteriologists, and the investigations of the subject have led to very far-reaching results of both theoretical and practical importance. Without attempting to enumerate in full the different properties which blood serum has been found to possess in its various reactions with bacteria and their products, those properties which bear upon the present investigation may be briefly stated as follows:

The blood serum of many healthy animals when drawn into test-tubes has the power of destroying a larger or smaller number of bacteria when these are introduced into it. Thus large numbers of anthrax bacilli are destroyed by rabbit's blood serum in test-tube experiments. Typhoid, colon, and Asiatic-cholera bacteria are also destroyed by the blood serum of rabbits.

The same serum is not equally potent for different bacteria, and the serum from different animals of the same or of different species varies in bactericidal potency for the same organism. The chemical reaction of the serum seems to exert some effect upon this quality of the serum. The more alkaline the serum the more potent is its action apparently, and consequently the venous blood has been found sometimes to furnish more potent serum than the arterial blood from the same animal.[9 a]

The injection of animals with the products of growth of bacteria produces different effects upon the properties of the blood serum, depending upon the kind of bacteria employed for injection. The injection of the bacteria of the group to which *B. choleræ suis*, *B. typhosus*, and *B. coli communis* belong appears sometimes to have no effect upon

[a] The figure references refer to bibliography at end of bulletin.

the bactericidal potency of the serum. At other times it seems to increase the potency, but frequently it has a very peculiar effect, for in some cases the serum after the injection of the animal will no longer kill any of the bacteria of the kind used to inject the animal, but on the dilution of the serum with an indifferent fluid, such as salt solution, it becomes strongly bactericidal for the bacteria of this kind. But this serum behaves like the serum from an uninoculated animal with other bacteria than those with which the animal is inoculated. Thus the serum from the blood of a rabbit injected with typhoid bacilli will often fail to kill the typhoid bacilli unless the serum is greatly diluted, but it will kill just as many Asiatic-cholera spirilla as it did before the animal was injected with the typhoid bacilli.[4] The peculiar behavior of the serum from an animal injected with a culture of bacteria, in its failure to kill the species of bacteria with which the animal is injected unless it is diluted, is called the Neisser-Wechsberg[14] phenomenon, after the two investigators who first observed the reaction.

DEFINITION OF SERA USED IN EXPERIMENTS.

The serum obtained from an animal which has been injected with bacteria is spoken of as immune serum in contradistinction to the serum from an uninoculated animal, which is called normal or fresh serum. Thus serum obtained from a hog injected with *B. choleræ suis* would be known as *B. choleræ suis* immune hog serum. So wherever immune serum is mentioned in the present paper it is to be understood that it refers to the serum obtained from an animal which has received at least one injection of bacteria, but not necessarily that the serum has either immunizing or germicidal properties. On the other hand, where normal or fresh serum is mentioned, it is to be understood to refer to serum obtained from an uninoculated animal.

Serum loses in bactericidal potency on standing after being drawn from the animal; and the higher the temperature to which the serum is exposed the more rapid the loss of potency. It may remain potent for several days or for even a week or more in the refrigerator, but if kept at body temperature it generally loses all bactericidal properties in three or four hours. Heating at 55° or 56° C. for ten or fifteen minutes also robs the serum of its bactericidal power, or rather this treatment of immune serum suspends its bactericidal power, which is restored by the addition of a small amount of fresh serum. This suspension of bactericidal power by heating at 55° or 56° C. is called inactivating the serum, and wherever inactivated serum is mentioned it is to be understood to refer to serum which has been treated in this way. Inactivated serum to which fresh serum is added, and which has had its bacteriolytic properties restored in this way, is termed reactivated serum.

NATURE OF THE SUBSTANCE CAUSING DESTRUCTION OF BACTERIA.

In regard to the nature of the substance or substances in the serum which cause the destruction of the bacteria which are introduced, there is some difference of opinion. Some authorities maintain with Buchner that there is only one substance, called by him "alexin," which causes bacteriolysis, while others—and these appear to be in the majority—maintain that there are two substances concerned. Those who hold to the former view regard the effect produced by standing or by heating as due to the weakening of the alexin, while those holding the latter view explain this effect by a modification of one of the two substances which they regard as necessary for bacteriolysis. All are not agreed, however, as to the nature of these two substances. Bordet [1,2,3] and the French school generally look upon the substance which is not modified by heat as a sensitizing agent merely, which acts upon the bacteria in such a manner as to make them susceptible to the destructive action of the other body to which the bacteriolytic properties of the serum are directly due. Bordet borrows Buchner's term "alexin" for this active agent, and he gives to the other body the name "substance sensibilisatrice." According to the French school, then, bactericidal serum owes its power to two substances, called, respectively, substance sensibilisatrice and alexin. The former resists heating up to 75° C. or even somewhat higher temperatures for an hour or more, while the latter is destroyed by heating at 55° C. or higher in ten or fifteen minutes. Moreover, the substance sensibilisatrice according to this view is a specific substance in each case. The substance sensibilisatrice for one kind of bacterium sensitizes this one kind only, and while it can not of itself cause bacteriolysis, it nevertheless produces certain changes in the bacteria. On the other hand, according to the view of Ehrlich and his school, this body, the thermostabile body, causes no change in the bacteria themselves, but serves merely as an intermediary, a binding link, serving to connect the bacteria to the active body—the alexin of Bordet—which according to this view is the active agent in bacteriolysis. To the two hypothetical bodies concerned in the process Ehrlich has given, at different times, different names. At first he called the thermostabile body "intermediary body," afterwards "immune body," and finally "amboceptor." The last two designations are those now generally employed by the Ehrlich school for the body which corresponds, as is evident, to the substance sensibilisatrice of Bordet. To the other body, the alexin of the French school, Ehrlich has given variously the names "addiment" and "complement," the last being that now exclusively employed. The term intermediary body has been employed in a recent publication for the amboceptors present in the normal serum in order to distinguish these

from immune amboceptors, or amboceptors produced by injecting animals with bacteria or their products.

But among those who have accepted more or less completely the Ehrlich conception of the nature of the bodies concerned in bacteriolysis there is difference of opinion as to the mode of action of the complement. Ehrlich himself says in regard to the matter "that one will not go amiss if he assumes with Pfeiffer that the process of bacteriolysis is analogous to digestion, and attributes to the addiment (complement) the character of a digestive ferment." Gruber,[8] on the contrary, contends that the complement does not act like a ferment, and that it is erroneous to draw any analogy between the complement and an enzym, since the complement is entirely used up in bacteriolysis, whereas in the process of fermentation, as is well known, the ferment is not used up, but may be recovered after the action is ended, and used for the fermentation of other material.

But whatever the exact mode of action may be, it is evident from what has just been said that both the Ehrlich and the Bordet schools attribute bacteriolytic action proper in normal serum to a substance easily changed by comparatively low temperatures, and called, respectively, complement and alexin by the two schools. To the other body concerned in bacteriolysis—the amboceptor of Ehrlich and the substance sensibilisatrice of Bordet—is assigned by the former the rôle of a binding link between the complement and the bacterium, while by the latter is assigned to it the property of a sensitizer, or of a mordant as in dyeing. In the one case the bacterial cell is regarded as not at all injured or otherwise changed by the union with the amboceptor; in the other case it is the opinion of those holding this view that the cell is acted upon and changed by the sensibilisatrice in such a way that the alexin can penetrate it.

Bordet[2] summarizes the difference between his theory and that of Ehrlich as follows:

> According to Ehrlich and Morgenroth the specific antibody (sensibilisatrice) plays the rôle of an actual intermediary (zwischenkörper, amboceptor), a link of union attaching itself on the one hand to the cell, on the other to the alexin. In other words, the absorption which the alexin undergoes in the presence of the sensitized cell is not due to an affinity manifested by the cell itself to this substance. The absorption of the alexin is only indirect; the cell joins itself to the intermediary substance, which is itself united chemically by its other pole to the alexin.
>
> Our idea of the phenomenon, which we feel we are justified in holding, is altogether different from this. To us it seems that the sensibilisatrice which unites with the cell modifies this in a way which permits it to absorb the alexin directly. The action of the sensibilisatrice upon the cells is comparable to that of certain fixative agents or mordants which confer upon certain substances (or to the cells of these substances, as is the case in histological technic) the power of absorbing colors which they refuse to absorb before treatment. * * * It is to be clearly understood, however, that when we speak of mordants in this connection we do not intend to apply in all details the phenomena of dyeing to the matter at present under consideration; we merely mean to draw a comparison which will serve to make our

idea clearer. The hypothesis which we wish to bring out in relief is that in the presence of hemolytic serum, the cell becomes capable of absorbing directly the alexin by means of its own proper elective affinity, and that this power is due to a change caused by the sensibilisatrice. In other words, we do not believe that one is forced to admit, with Ehrlich and Morgenroth, that the sensibilisatrice itself combines with the alexin, and that this union is indispensable for the latter substance to attack the cell.

Bordet furthermore states in the same connection that he agrees with Buchner in regarding the alexin for blood cells and for bacteria as identical—that one and the same alexin may attack the most diverse cells; whereas Neisser and others of the Ehrlich school believe that alexins or complements are different in one and the same serum.

While it is evident from the above that the terms amboceptor and sensibilisatrice are used to designate the same substance, it is scarcely correct to use them interchangeably, since they connote somewhat different attributes in the body to which they refer. The same is true of the terms complement and alexin, though to a less degree.

The following diagrams, obtained from various sources and modified to suit the purpose, will serve to illustrate the process of bacteriolysis according to the views of the Ehrlich school.

Figure 1 represents in its simplest form the mechanism of bacteriolysis according to the Ehrlich hypothesis, and serves to illustrate the process sufficiently for the purposes of the present paper.

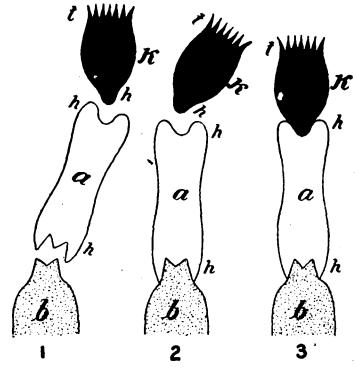

FIG. 1.—Illustration of the mechanism of bacteriolysis according to the Ehrlich hypothesis.

In the diagram the bacteria are represented by the parts marked *b*, the amboceptors by those marked *a*, and the complements by those marked *k*. In No. 1 the bacterium, amboceptor, and complement are represented as just on the point of uniting. No. 2 represents the bacterium and the amboceptor united and the complement on the point of uniting with the unoccupied end of the amboceptor. No. 3 represents the process of uniting of bacterium, amboceptor, and complement completed; the bacterium in this case would undergo bacteriolysis.

It should be borne in mind that according to this theory bacteriolysis can take place only where the bacterium becomes united to an amboceptor which is itself united with a complement. A bacterium may become united with a free amboceptor—i. e., an amboceptor which is not united with a complement—but the bacterium in such a case does not undergo bacteriolysis unless a complement subsequently becomes attached to the amboceptor. The complement is incapable of uniting directly with a bacterium; it can do this only through the

intervention of the amboceptor. But when the complement becomes linked to the bacterium by means of the amboceptor the bacterium becomes broken up into minute granules and ultimately disappears.

The bonds by which the amboceptor attaches itself to the bacterium on the one hand, and to the complement on the other, are called haptophor groups or haptophors (*h*), and similarly this name is given to the bonds of union of the bacteria and of the complement. The amboceptor thus has two haptophors, one by means of which it attaches itself to the bacterium, the cytophylic haptophor, and one by means of which it attaches itself to the complement, the complementophylic haptophor. The bacteria probably possess each many haptophors all of the same kind—i. e., haptophors capable of uniting with amboceptors of the same kind—but for the sake of simplicity the bacterium is represented in the diagram as having only one haptophor. The complement has one haptophor group and one so-called toxophor group (*t*), and it is by means of the latter group that the complement acts upon the bacterium. The complement may be deprived of this toxophor group, and although it is still capable of uniting with the amboceptor in such a case, it can no longer cause bacteriolysis. This loss of the toxophor group is caused by heating, and it also occurs spontaneously in the serum on standing. Bacteria subjected to the action of heated serum do not undergo bacteriolysis, but become fixed to the amboceptors, and the amboceptors become united to the haptophor group of the complement which are left unaffected by the heating. It will thus be readily understood why bacteria treated with heated immune serum are subsequently protected from bacteriolysis even when unheated immune serum or when unheated complement is added to them. The complementophylic haptophor of the amboceptor is in such a case already occupied by the haptophors of the heated complement, which has in this way become deprived of its toxophor group.

The amboceptors found in ordinary normal serum are either all alike—and in this case they must possess affinity for a great many different kinds of bacteria—or they must differ from one another; and in this case there must evidently be a great many specific amboceptors, some fitted for the bacteriolysis of one species of bacterium, some for others. This matter seems not yet to have been settled. But it is certain that the injection of an animal with certain bacteria or their products causes the formation of a large number of specific amboceptors; that is to say, of amboceptors having affinity only for the kind of bacteria with which the animal is injected. Such injections seem not to increase the amount of complement.

Complement is found normally in the serum, that of some animals possessing more than that of others. The horse appears to have a large amount of complement in the serum. It is not yet settled whether the complement is specific—that is, whether the complement for one kind of immune serum can unite with the amboceptors of this

serum only and not with the immune serum of a different sort—or whether complements are general; though they seem for the most part not to be specific. The complement in the serum of horse's blood seems capable of reactivating heated immune serum of various kinds. Still in some cases it would appear as if they were specific.

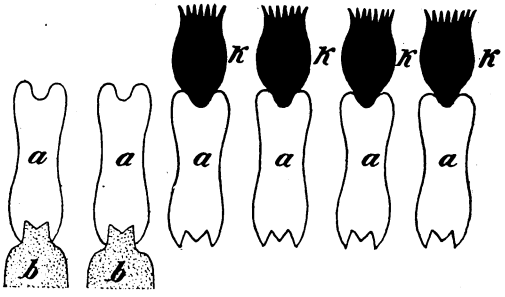

FIG. 2.—Diversion of complement in undiluted immune serum.

With the explanation given above of the nature of amboceptors and complements, the phenomena which take place in immune serum become more or less satisfactorily explicable. By means of the characteristics ascribed to these bodies it is possible to account for the peculiar behavior of immune serum stated above, consisting in the fact that such serum is frequently more potent when diluted than when it is undiluted.

Neisser and Wechsberg were the first to observe this phenomenon, and the theory which they advance to explain it they very appropriately call the theory of the diversion of complement. As the name implies, they attribute the lack of bacteriolysis in the undiluted immune serum to the turning aside of the complement from the bacteria, or rather from the amboceptors which are attached to the bacteria. They hold that this diversion is brought about by the free amboceptors themselves. In other words, where there are more amboceptors than there are complements present in a serum, a part of these attach themselves to the bacteria and a part to the complements.

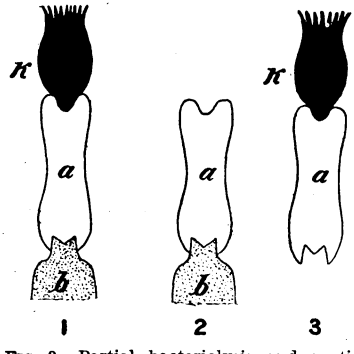

FIG. 3.—Partial bacteriolysis and partial diversion of complement in diluted immune serum

The accompanying diagram (fig. 2), taken from Neisser and Wechsberg, and modified to suit the present description, shows two amboceptors *a*, attached to bacteria *b*, and four amboceptors attached to complements *k*. Bacteriolysis is not possible in such a condition, because the complements have been diverted from the amboceptors which are attached to the bacteria. Bacteriolysis can take place only when the complement becomes attached to the bacterium through the medium of the amboceptor.

Figure 3 is meant to show the same serum diluted with an equal amount of salt solution. In this case, with the same number of bacteria added, it is evident that one-half of them would be killed, as

is indicated by the combination between bacteria, amboceptor, and complement in No. 1. The other half of the bacteria would evidently escape, the complement being diverted by the free amboceptor, as shown by Nos. 2 and 3.

Although these statements in regard to bacteriolysis and the mechanism probably involved are by no means exhaustive, they will, perhaps, serve the purposes of the present investigation and to explain the results here obtained. It will be noticed that experiments have been made with a view to throwing additional light upon those phases of bacteriolysis already mentioned, as they were observed in hog's blood serum, and it would appear that the results, although differing in some instances from those of other observers, are nevertheless susceptible of interpretation in harmony with more or less firmly established hypotheses.

METHODS USED IN DRAWING BLOOD, AND DESCRIPTION OF CULTURES EMPLOYED.

The blood was drawn from healthy hogs, or from hogs injected with cultures of *B. choleræ suis*, subcutaneously or intravenously, as stated in each case, at various lengths of time before the drawing. It was obtained (1) by cutting off a piece of the tail and allowing the blood to flow into sterilized tubes or flasks, (2) by bleeding from an artery in the ear, or (3) by inserting a canula into the carotid artery or jugular vein. In some cases the blood was drawn from the carotid and from the jugular at the same operation. After drawing, the blood was usually placed in the refrigerator to allow the serum to separate, but in a few cases it was used immediately after drawing.

All the hogs used in the experiments were in a perfectly healthy condition to start with, and, as far as could be ascertained, had never been sick. They weighed from 30 to 40 pounds each.

The cultures employed were obtained during the course of former experiments. One of them, G. P. 4692, had been repeatedly passed through guinea pigs; another, Crawford, had been carried along for a number of years on artificial media without passage through animals; the third, F. 26, had also been carried along for several years on artificial media without animal passage. These cultures presented minor points of difference from one another, but they were all quite typical for *B. choleræ suis*. All of them give characteristic growths upon artificial culture media with the usual fermentation reactions of the sugars, and the other features of *B. choleræ suis*, both macroscopic and microscopic, though it is true that the Crawford strain grew more vigorously and gave larger and denser colonies than the other two, and that the G. P. 4692 strain gave the least vigorous growth on artificial media. Also, in the tests which were made to determine the point, the G. P. 4692 strain was much more strongly pathogenic for

guinea pigs, rabbits, and hogs than the other two strains. The Crawford strain was the least virulent of the three. So the virulence of the three organisms was in inverse ratio to the vigor of growth upon artificial culture media. The culture of *B. coli communis* was obtained in the course of former experiments from the feces of a normal hog. It showed no pathogenic properties.

THE BACTERICIDAL POTENCY OF SERUM FROM THE SAME HOG AT DIFFERENT TIMES.

The bactericidal potency of the serum from the same hog on different days was tested by bleeding a hog at intervals of a few days, drawing off the serum after the blood had stood in the refrigerator for twenty-four hours or less, and distributing it into test tubes—1 c. c. into each tube—then adding a definite, measured amount of a suspension in salt solution of the bacteria to be tested. At the same time a tube of 1 c. c. of salt solution was inoculated with the same measured amount of bacterial suspension and plates made immediately to determine the number of bacteria added. Plates were made from the serum tubes on the following day. The serum from 4 hogs was used in this experiment, the blood in all four experiments being drawn from the tail. The results will be found in the following tables:

TABLE I.—*Bacteriolytic action of normal hog serum from the same animal at different drawings. Blood drawn from the tail. Hog No. 1733.*

Date.	Time after drawing.	No. of drawing of blood.[a]	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.					
April 7	3 hours	I	G. P. 4692	4,620	79,800
7	do	I	*B. coli*	5,880	30,800
9	do	II	G. P. 4692	30,800	48,300
9	do	II	*B. coli*	7,000	0
11	1 day	III	G. P. 4692	4,160	9,800
11	do	III	*B. coli*	225	53
14	do	IV	G. P. 4692	9,800	4,690
14	do	IV	*B. coli*	9,520	9,800
19	do	V	G. P. 4692	8,655	142,900
19	do	V	*B. coli*	2,600	0
22	2 days	VI	G. P. 4692	4,968	43,200
22	do	VI	*B. coli*	1,940	240
26	1 day	VII	G. P. 4692	1,800	1,220
26	do	VII	*B. coli*	2,800	16,870
May 3	3 hours	VIII	G. P. 4692	22,628	17,510
3	do	VIII	*B. coli*	23,380	84
5	do	IX	G. P. 4692	8	1,004
5	do	IX	*B. coli*	869	2,594

[a] Roman numerals denote the serial numbers of the drawings of blood.

As will be seen from Table I, the blood was drawn from hog 1733 on nine different days and tested simultaneously upon cultures of G. P. 4692 and on the culture of *B. coli communis*. In four of the drawings the serum apparently had no bacteriolytic power for the strain of *B. choleræ suis* employed, while in four of them, although there was also no marked bacteriolysis, there was nevertheless no evidence of

any great increase of the bacteria introduced. In the serum from one of the drawings the bacteria were reduced to one-half of the number introduced; this was the greatest amount of potency shown in any of the drawings.

With the colon bacillus there was marked bacteriolysis in one drawing, less marked in four other drawings, and neither increase nor decrease in one of the drawings, while in the other three drawings there was an increase of the bacteria introduced.

It is evident that the serum from this hog showed different degrees of potency or absence of potency upon the different occasions when the blood was drawn.

TABLE II.—*Bacteriolytic action of normal hog serum from the same animal at different drawings. Blood drawn from the tail. Hog No. 1740.*

Date.	Time after drawing.	No. of drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.					
May 6	3 hours	I	G. P. 4692	4	1,750
6	.....do	I	*B. coli*	975	8
8	.....do	II	G. P. 4692	4,925	1,276
8	.....do	II	*B. coli*	3,778	70
9	.....do	III	G. P. 4692	102	1,236
10	.....do	IV	G. P. 4692	1,010	1,552
10	.....do	IV	*B. coli*	3,110	994
11	.....do	V	G. P. 4692	848	450
12	.....do	VI	G. P. 4692	138	0
12	.....do	VI	*B. coli*	1,700	1,840

The blood of hog 1740, as is shown in Table II, was drawn on six different days and tested on *B. coli* and on G. P. 4692. It showed marked bactericidal power for the *B. coli* culture in three of the four drawings tested with this organism, but in one there was no decrease in the number of bacteria introduced. With the *B. choleræ suis* culture it will be noticed from the table that the effect varied greatly with the different drawings. While there was no very marked decrease in any case, it is evident in general that the bacteria did not multiply abundantly in the serum in any case. It would seem as if growth had been inhibited by the serum, although active bacteriolysis was lacking except in the serum from one drawing.

TABLE III.—*Bacteriolytic action of normal hog serum from the same animal at different drawings. Blood drawn from the tail. Hog No. 1741.*

Date.	Time after drawing.	No. of drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.					
May 8	3 hours	I	G. P. 4692	4,925	82
8	.....do	I	*B. coli*	3,778	14
9	.....do	II	G. P. 4692	102	166
9	.....do	II	*B. coli*	1,970	390
10	.....do	III	G. P. 4692	1,010	1,932
10	.....do	III	*B. coli*	3,110	40
11	.....do	IV	G. P. 4692	848	50
12	.....do	V	*B. coli*	1,700	0

As Table III shows, the blood was drawn from hog 1741 on five different occasions. The serum from four of the drawings was tested upon the culture of *B. coli*, and it showed marked bacteriolytic properties for this organism in all the tests. The serum from four of the drawings was tested upon the G. P. 4692 strain of *B. choleræ suis*, but only two of the drawings showed bacteriolytic properties for this organism.

TABLE IV.—*Bacteriolytic action of normal hog serum from the same animal at different drawings. Blood drawn from the tail. Hog No. 1742.*

Date.	Time after drawing.	No. of drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c.c.	Number of bacteria per 1 c c. after 1 day.
1905.					
May 8	3 hours	I	G. P. 4692	4,925	82
8	do	I	*B. coli*	3,788	686
10	do	II	G. P. 4692	102	46
10	do	II	*B. coli*	1,970	82
12	do	III	G. P. 4692	1,010	798
12	do	III	*B. coli*	3,110	30
11	do	IV	G. P. 4692	848	50
13	do	V	*B. coli*	1,700	0
23	do	VI	G. P. 4692	10,880	3,376
23	do	VI	Crawford	40,500	2,640
23	do	VI	F. 26	19,040	18,280
24	do	VII	G. P. 4692	1,682	0
24	do	VII	Crawford	1,808	180
24	do	VII	F. 26	12,040	1,640
26	do	VIII	Crawford	320	0
26	do	VIII	F. 26	80	1,960
29	do	IX	F. 26	1,740	4,480
29	do	IX	Crawford	2,320	0
29	do	IX	G. P. 4692	1,920	960
June 5	½ hour	X	F. 26	2,840	26,640
5	do	X	Crawford	3,380	180
5	do	X	G. P. 4692	1,380	4,420

In the experiments with the blood of hog 1742, as shown in Table IV, ten different drawings were made on separate days, and four of them tested on the culture of the colon bacillus, eight on culture G. P. 4692, five on culture Crawford, and five on culture F. 26.

The colon bacillus was greatly diminished in all cases. In one case where 1,700 per 1 c. c. had been introduced into the serum all of them were destroyed, and in another test 3,000 out of 4,000, in round numbers, were killed.

The tests with the G. P. 4692 culture showed that the serum was bactericidal in seven tests out of eight. In one of these about 1,700 bacilli were introduced and all of them were killed. On the other hand, in the serum from one of the drawings there was an increase of the bacilli introduced.

The tests with the Crawford culture showed that the serum from all of the drawings tested was actively bactericidal, but in some more than in others. In one of the drawings about 38,000 bacilli per 1 c. c. of serum were destroyed out of the 40,000 introduced. But in another case all were not destroyed although only 1,808 were introduced.

Tests with culture F. 26 showed bacteriolysis in one drawing; in another drawing there was no increase nor decrease of the bacteria introduced. In three drawings there was marked increase of the organisms introduced.

That the bactericidal potency of the serum of hogs should vary at different times, as these results seem to indicate, should perhaps not be a matter of surprise. There are probably many circumstances which influence this property of the serum. As is stated elsewhere in this paper, the chemical reaction of the serum has been found by others to influence the bactericidal power, and doubtless there are other as yet obscure circumstances which raise or lower this power of the serum. The nature of bacteriolysins will be found discussed at some length below in a different connection, and it would not seem at all improbable that there may be more of these at one time than at another present in the blood serum. Indeed, the production of bacteriolysis in serum of the living animal seems to be easily influenced one way or another, and it would not be unreasonable to regard them as varying from time to time under even slightly changing conditions of the body.

Trommsdorf[19] noticed that human sera derived from normal individuals as well as from those suffering from various diseases vary greatly in bacteriolytic power. Petterson[15] found the same thing with chickens.

Morgenroth and Sachs[13] found great variation in cytolytic power in serum of various sorts. Thus the serum from a horse at one drawing was hemolytic for rabbits' corpuscles but not for those of guinea pigs; three days later the serum from the same horse was strongly hemolytic for guinea pigs' corpuscles, but only very slightly for rabbits' corpuscles; twenty-three days later the serum from this horse was not hemolytic for guinea pigs' corpuscles, but strongly hemolytic for rabbits' corpuscles.

It is therefore evident that the cytolytic power of serum is very variable. Not only does the blood from different individuals of the same species differ in this respect, but the serum from the same individual differs from time to time. This is probably the case with all animals, and, as is apparent in the experiments with hogs, these are no exception to the rule.

In regard to the above experiments, while there is more or less variation between single tests, if one strain only of *B. choleræ suis* is considered, there seems to be a general tendency toward either active bacteriolysis or else inhibition of growth.

MODIFICATIONS WHICH OCCUR IN THE BACTERICIDAL POWER OF SERUM UPON STANDING.

As has been previously stated, the blood was placed on ice in all cases where it was not at once used for making tests. In some cases the bactericidal properties of the serum were tested after the serum had stood for various lengths of time. The results of these tests, as will be seen from the accompanying tables, show in general that the bactericidal potency is retained in some cases for as long as nine days. Occasionally, however, the bactericidal power of the serum is diminished by even two days' standing.

TABLE V.—*Bacteriolytic action of normal hog serum after standing for various lengths of time in the refrigerator. Blood drawn from the tail, eighth drawing. Hog No. 1742.*

Date.	Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.				
May 26	3 hours	Crawford	320	0
June 2	7 days	do	1,960	380
May 26	3 hours	F. 26	80	1,960
June 2	7 days	do	3,280	2,980

The serum from the blood of hog 1742 at the eighth drawing, as is shown in Table V, was tested upon two of the organisms only, namely, Crawford and F. 26. For the Crawford culture the bactericidal power of the serum was retained for seven days, whereas for the F. 26 culture the serum was bactericidal at the start and only inhibitory after seven days.

TABLE VI.—*Bacteriolytic action of normal hog serum after standing for various lengths of time in the refrigerator. Blood drawn from the tail, ninth drawing. Hog No. 1742.*

Date.	Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.				
May 29	3 hours	F. 26	1,740	4,480
June 3	5 days	do	1,220	1,180
May 29	3 hours	Crawford	2,320	0
June 3	5 days	do	1,060	0
May 29	3 hours	G. P. 4692	1,920	960
June 3	5 days	do	700	1,100

At the ninth drawing from hog 1742, as Table VI shows, the serum retained its bactericidal potency for the Crawford culture apparently unabated for five days, whereas for the other two organisms there was apparently more or less inhibition of growth, perhaps, both at the start and after five days, but no very marked bacteriolysis for either of them.

TABLE VII.—*Bacteriolytic action of normal hog serum after standing for various lengths of time in the refrigerator. Blood drawn from the tail, tenth drawing. Hog No. 1742.*

Date.	Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.				
June 5	½ hour	F. 26	2,840	26,640
6	1 day	do	2,240	51,760
7	2 days	do	3,440	900
8	3 days	do	2,200	1,340
9	4 days	do	1,360	18,280
19	14 days	do	4,040	a ∞
5	½ hour	Crawford	3,380	180
6	1 day	do	5,200	120
7	2 days	do	7,440	760
8	3 days	do	3,500	3,160
9	4 days	do	2,640	1,380
19	14 days	do	1,440	∞
5	½ hour	G. P. 4692	1,380	4,420
6	1 day	do	3,040	6,320
7	2 days	do	180	3,420
8	3 days	do	360	2,000
9	4 days	do	1,880	∞
19	14 days	do	1,740	∞

a This character signifies that there were too many colonies to count.

The serum from the blood at the tenth drawing from hog 1742, as Table VII shows, preserved its bactericidal power to a marked degree for the Crawford culture up to and including the second day of standing. It appears to have been somewhat more potent on the fourth than on the third day of standing, but the difference in potency of the serum on these two days is hardly sufficient, perhaps, to be of any great significance. For the other two strains there was at most inhibition shown in some of the tests, but no decided bacteriolysis either before or after standing, except perhaps in the case of the F. 26 strain after two days. But in the case of this organism the serum appeared to have been more potent after standing for three days than it was previous to this time.

TABLE VIII.—*Bacteriolytic action of normal hog serum after standing for various lengths of time in the refrigerator. Blood drawn from the tail, eleventh drawing. Hog No. 1742.*

Date.	Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.				
June 12	½ hour	F. 26	2,580	650
16	4 days	do	1,000	1,180
19	7 days	do	4,040	16,500
22	10 days	do	26,000	a ∞
12	½ hour	Crawford	2,560	400
16	4 days	do	2,860	280
19	7 days	do	1,440	320
22	10 days	do	50,400	∞
12	½ hour	G. P. 4692	15,120	800
19	7 days	do	1,740	1,280
22	10 days	do	8,880	∞

a This character signifies that there were too many colonies to count.

The serum from the blood of hog 1742 at the eleventh drawing, as shown in Table VIII, had marked bactericidal power for all three strains at the start, and this was retained for as long as seven days for the Crawford culture, whereas there was inhibition only for F. 26 after four days, and after this there was not even inhibition for this organism. The effect upon the G. P. 4692 culture was similar to that upon the F. 26 strain.

TABLE IX.—*Bacteriolytic action of normal hog serum after standing for various lengths of time in the refrigerator. Blood drawn from the jugular vein, twelfth drawing. Hog No. 1742.*

Date.	Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c.c.	Number of bacteria per 1 c.c. after 1 day.
1905.				
June 22	½ hour	Crawford	4,320	a ∞
23	1 day	do	4,060	∞
22	½ hour	F. 26	3,380	∞
23	1 day	do	640	∞
22	½ hour	G. P. 4692	1,240	∞
23	1 day	do	340	∞

a This character signifies that there were too many colonies to count.

The serum from the blood of hog 1742 at the twelfth drawing (Table IX) was taken from the jugular vein, and it will be observed from the table that the serum showed no bacteriolytic power either at the start or on standing. In order to test whether the lack of bactericidal power was due in this case to the fact that the blood was drawn from the vein, experiments, which are hereinafter given (page 22), were made to compare the bactericidal power of arterial blood serum with that of venous blood serum.

COMPARISON OF THE BACTERICIDAL POTENCY OF THE SAME SERUM FOR DIFFERENT STRAINS OF BACTERIA.

If in the foregoing tables comparison is made between the results obtained with the same drawing of blood upon the various organisms, it will be seen that quite decided differences appear.

Selecting the most striking contrasts, in one case there was a decided increase of the *B. choleræ suis*, culture G. P. 4692, while in the same blood 7,000 *B. coli* per 1 c. c. were all destroyed. It is true that in this case a great many more of *B. choleræ suis* were introduced at the start than of *B. coli*, and this may have influenced the result somewhat. But in another case where approximately the same number of organisms of the two kinds were introduced at the start there were very few of the *B. choleræ suis*, if any, destroyed, whereas nearly all of the *B. coli* were destroyed. In still another case there was a very great increase of *B. choleræ suis*, while all of the *B. coli* were destroyed. In one case there was no notable increase of the *B. choleræ suis*, while there was a decrease of the *B. coli*.

It is evident, therefore, that the bactericidal power of hog serum is different for this strain of *B. choleræ suis* and for the *B. coli*, in so far as this can be determined by the methods at present in use.

THE POTENCY OF SERUM FROM ARTERIAL BLOOD COMPARED WITH THAT FROM VENOUS BLOOD.

As hereinbefore stated, the following experiments were undertaken because of the results obtained with the serum from the venous blood in the experiment in which it appeared that there was neither inhibitory nor bactericidal power in the serum. In the experiments previous to this one the blood was drawn from the tail, and was consequently mainly arterial. It spurted from a severed artery when the tail was cut. In the experiments at present under consideration the blood was drawn from the carotid artery or from the jugular vein either at the same operation or on different occasions.

TABLE X.—*Bacteriolytic action of arterial serum compared with that of venous serum, both sera from the same normal hog (No. 1733) at the same operation. Tenth drawing of blood. Tests made at intervals after drawing.*

Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.	
			Arterial serum.	Venous serum.
1 day	F. 26	3,880	60	320
2 days	do	4,920	16,320	600
9 days	do	380	2,200	400
1 day	Crawford	940	40	80
2 days	do	1,480	200	120
9 days	do	420	140	40
1 day	G. P. 4692	2,440	9,760	5,920
2 days	do	9,880	8,160	6,560
9 days	do	300	100	140

The serum from hog 1733 at the tenth drawing, as shown in Table X, was taken at the same operation from the carotid artery and from the jugular vein. In the tests with the Crawford culture the serum from the arterial, as well as that from the venous blood, seem both to have possessed considerable potency, and to have possessed it in about equal degree. They furthermore appear to have retained their potency, much or all of it, for nine days. For G. P. 4692 culture neither serum appears to have had strong bactericidal power in any case. They both seem to have had some inhibitory power for this organism both at the start and after nine days. For culture F. 26 there appears to have been no difference between the two kinds of serum after having stood for one day, but the serum from the venous blood would seem to have retained its potency upon standing more tenaciously than that from the arterial blood.

TABLE XI.—*Bacteriolytic action of arterial serum compared with that of venous serum, both sera from the same normal hog (No. 1733) at the same operation. Eleventh drawing of blood. Tests made at intervals after drawing.*

Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.	
			Arterial serum.	Venous serum.
1 day	F. 26	5,420	10,480	220
2 days	do	6,520	20,160	27,680
5 days	do	8,580	280	8,960
8 days	do	240	10,960	2,600
1 day	Crawford	8,160	160	10,860
2 days	do	8,660	400	360
5 days	do	26,240	20,640	580
8 days	do	1,920	7,280	60
1 day	G. P. 4692	2,160	2,520	3,300
2 days	do	2,720	2,960	3,340
5 days	do	a ∞	21,240	1,360
8 days	do	2,240	∞	2,320

a This character signifies that there were too many colonies to count.

The blood obtained at the eleventh drawing from hog 1733, as shown in Table XI, was drawn from the artery and from the vein at the same operation. There seems to have been a difference in the potency between the two kinds of serum for all three organisms. For the Crawford culture the serum from the arterial blood seems to have been quite strongly bactericidal at the start, but the serum from the venous blood was apparently not so. The tests made after the serum had stood for two days would indicate that both kinds of serum were potent. The serum from the venous blood in this case seems to have gained in potency upon standing, a result previously noted in the case of culture F. 26 with a different serum, but it seems necessary to conclude that an error of some kind must have crept in where such results are obtained, since they are at variance not only with the a priori probabilities of the case but also with the usual experience. On the whole, the results in this experiment seem to indicate for the Crawford culture a greater potency, or at least a retention of a greater amount of potency, on the part of the venous than of the arterial blood.

For culture F. 26 in this experiment the arterial serum appeared less potent at the start and after eight days than the venous serum, but after standing five days it was more potent than the venous serum. Here again the serum seems to have gained potency on standing.

For G. P. 4692 the stronger retention of potency of the venous serum as compared with the arterial is apparent in the tests made five days and eight days after drawing. No difference between the two sorts of serum is noticeable in the tests made with this organism one day and two days after drawing.

The fact that blood through which a stream of CO_2 was conducted becomes more alkaline led Hamburger[9] to make tests of the comparative bactericidal potency of serum from ordinary blood and that from

blood through which CO_2 had been passed, as well as of the difference in this respect between arterial and venous blood. He found, in fact, that serum from venous blood and from blood through which CO_2 had been passed is more potent than arterial-blood serum.

EFFECTS OF HEAT UPON THE BACTERICIDAL POWER OF HOG SERUM.

A large number of tests were made parallel with those above recorded to determine the effect of heat upon the germicidal power of hog serum, and also to determine whether there is any difference between the potency of the heated serum for the different strains of *B. choleræ suis* upon which it was tested.

The method employed in making the tests consisted in immersing the tubes containing the serum up to the plugs in a water bath which was kept by means of a thermoregulator exactly at the temperature to be tested. The results are shown in the following tables, from which it will be seen that the serum was subjected to temperatures of 51°, 52°, 53°, and 54° C. for ten minutes and for thirty minutes at different times. Tests were also made at temperatures above 54° C., but these are not tabulated for the reason that they gave results similar to those at 54°.

TABLE XII.—*Effects of heat upon the bacteriolytic power of normal hog serum. Blood drawn from the tail and kept in the refrigerator for various lengths of time. Temperature employed, 51° C.*

Date.	Time after drawing.	No. of hog and drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Arterial blood. Number of bacteria per 1 c. c. after 1 day.		Length of exposure to heat.
					Not heated.	Heated.	
1905.							
April 7	3 hours	1733 I	G. P. 4692	4,620	70,800	49,000	10 minutes.
	do	do	*B. coli*	5,880	30,800	36,400	Do.
June 12	½ hour	1742 XI	F. 26	2,850	650	96,600	30 minutes.
	4 days	do	do	1,000	1,180	1,160	Do.
	7 days	do	do	4,040	16,500	[a] ∞	Do.
	10 days	do	do	26,000	∞	∞	Do.
	½ hour	do	Crawford	2,560	400	440	Do.
	4 days	do	do	2,860	280	4,440	Do.
	7 days	do	do	1,440	320	4,200	Do.
	10 days	do	do	50,400	∞	∞	Do.
	½ hour	do	G. P. 4692	15,120	800	11,200	Do.
	7 days	do	do	1,740	1,280	1,840	Do.
	10 days	do	do	8,880	∞	28,560	Do.

[a] This character signifies that there were too many colonies to count.

It would appear from the results recorded in Table XII that heating for ten minutes at 51° C. produced no effect in the two tests which were made in this manner, one on the G. P. 4692 strain of *B. choleræ suis*, and the other on the colon culture. The same temperature for thirty minutes robbed the serum of much or all of its power in 8 of the 11 tests.

TABLE XIII.—*Effects of heat upon the bacteriolytic power of normal hog serum. Blood drawn from the tail. Temperature employed, 52° C.*

Date.	Time after drawing.	No. of hog and drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.				Length of exposure to heat.
					Arterial blood.		Venous blood.		
					Not heated.	Heated.	Not heated.	Heated.	
1905.									
June 28	1 day	1733 X	F. 26	3,880	60	1,540	320	10,720	30 minutes.
	2 days	do	do	4,920	16,320	15,200	600	3,360	Do.
	9 days	do	do	380	2,200	1,720	400	a ∞	Do.
	1 day	do	Crawford	940	40	3,960	80	25,080	Do.
	2 days	do	do	1,480	200	460	120	∞	Do.
	9 days	do	do	420	140	300	40	∞	Do.
	1 day	do	G. P. 4692	2,440	9,760	1,920	5,920	3,080	Do.
	2 days	do	do	9,880	8,160	9,840	6,560	10,000	Do.
	9 days	do	do	300	100	100	140	.....	
July 12	1 day	1733 XI	F. 26	5,420	10,480	.....	220	5,680	Do.
	2 days	do	do	6,520	20,160	14,880	27,680	41,720	Do.
	1 day	do	G. P. 4692	2,160	2,520	2,440	3,300	2,240	Do.
	2 days	do	do	2,720	2,960	2,180	3,340	(?)	

a This character signifies that there were too many colonies to count.

It will be seen from the above table that the effect of an exposure of thirty minutes showed a marked influence in some cases while in others it was apparently without effect. In these experiments the blood was drawn at the same operation from the carotid artery and from the jugular vein, and it would appear that the serum from the venous blood was somewhat more sensitive to the action of the heat than the serum from the arterial blood, if any conclusions are permissible from the somewhat limited number of observations. In the arterial there was in only one case a marked difference between the potency of the heated and of the unheated serum out of the 12 cases in which it was tried. In the serum from the venous blood, on the other hand, there was a marked difference between the heated and the unheated serum in the 11 tests which were made. On the whole the effect of heating at 52° C. would appear to be uncertain; sometimes heating seems to have a marked effect upon the bactericidal power of the serum; at others it seems to lack this effect.

TABLE XIV.—*Effects of heat upon the bacteriolytic power of normal hog serum. Blood drawn from the tail. Temperature employed, 53° C.*

Date.	Time after drawing.	No. of hog and drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.		Length of exposure to heat.
					Not heated.	Heated.	
1905.							
Apr. 19	1 day	1733 V	G. P. 4692	8,655	142,900	49,000	10 minutes.
19	do	do	*B. coli*	2,600	0	37,800	Do.
22	2 days	1733 VI	G. P. 4692	4,968	43,200	26,600	Do.
22	do	do	*B. coli*	1,940	240	2,100	Do.
26	1 day	1733 VII	G. P. 4692	1,800	1,220	2,988	Do.
26	do	do	*B. coli*	2,800	16,870	16,520	Do.
May 3	3 hours	1733 VIII	G. P. 4692	32,628	17,510	38,780	Do.
3	do	do	*B. coli*	23,380	84	9,920	Do.
5	do	1733 IX	G. P. 4692	8	1,004	13,720	Do.
5	do	do	*B. coli*	869	2,594	23,590	Do.
6	do	1740 I	G. P. 4692	4	1,750	89,600	Do.
6	do	do	*B. coli*	975	8	2,260	Do.
8	do	1740 II	G. P. 4692	4,925	1,276	7,380	Do.
8	do	do	*B. coli*	3,778	70	5,925	Do.
9	do	1740 III	G. P. 4692	102	1,236	1,900	Do.
10	do	1740 IV	G. P. 4692	1,010	1,552	3,559	Do.
10	do	do	*B. coli*	3,110	994	1,430	Do.
11	do	1740 V	G. P. 4692	848	450	2,354	Do.
12	do	1740 VI	G. P. 4692	138	0	1,550	Do.
12	do	do	*B. coli*	1,700	1,840	15,900	Do.
8	do	1741 I	G. P. 4692	4,925	82	4,144	Do.
8	do	do	*B. coli*	3,778	14	1,132	Do.
9	do	1741 II	G. P. 4692	102	166	910	Do.
9	do	do	*B. coli*	1,970	390	1,280	Do.
10	do	1741 III	G. P. 4692	1,010	1,932	1,778	Do.
10	do	do	*B. coli*	3,110	40	70	Do.
11	do	1741 IV	G. P. 4692	848	50	3,286	Do.
12	do	1741 V	*B. coli*	1,700	0	1,181	Do.
8	do	1742 I	G. P. 4692	4,925	.....	7,830	Do.
8	do	do	*B. coli*	3,788	686	17,580	Do.
10	do	1742 II	G. P. 4692	102	46	a ∞	Do.
10	do	do	*B. coli*	1,970	82	734	Do.
12	do	1742 III	G. P. 4692	1,010	798	2,412	Do.
12	do	do	*B. coli*	3,110	30	27,220	Do.
11	do	1742 IV	G. P. 4692	848	50	1,758	Do.
13	do	1742 V	*B. coli*	1,700	0	1,181	Do.
July 12	1 day	1733 XI b	F. 26	5,420	10,480	∞	30 minutes.
13	2 days	do	do	6,520	20,160	14,880	Do.
12	1 day	do	Crawford	8,160	160	33,500	Do.
13	2 days	do	do	8,660	400	16,720	Do.
12	1 day	do	G. P. 4692	2,160	2,520	2,440	Do.
13	2 days	do	do	2,720	2,960	2,180	Do.
12	1 day	1733 XI c	F. 26	5,420	220	5,680	Do.
13	2 days	do	do	6,520	27,680	41,720	Do.
12	1 day	do	Crawford	8,160	10,860	26,160	Do.
13	2 days	do	do	8,660	360	15,600	Do.
12	1 day	do	G. P. 4692	2,160	3,300	2,240	Do.
13	2 days	do	do	2,720	3,340	(?)	Do.

a This character signifies that there were too many colonies to count.
b Arterial blood serum.
c Venous blood serum.

As shown by the results recorded in Table XIV, heating at 53° C. had the effect in most cases of greatly diminishing the bactericidal power of the serum, though this is not to be noted in all cases, even where the serum was exposed for thirty minutes.

TABLE XV.—*Effects of heat upon the bacteriolytic power of normal hog serum. Blood drawn from the tail. Temperature employed, 54° C.*

Date.	Time after drawing.	No. of hog and drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.		Length of exposure to heat.
					Not heated.	Heated.	
May 26	3 hours	1742 VIII	Crawford	320	0	1,160	10 minutes.
June 2	7 days	do	do	1,960	380	2,380	Do.
May 26	3 hours	do	F. 26	80	1,960	5,280	Do.
June 2	7 days	do	do	3,280	2,980	9,360	Do.
May 29	3 hours	1742 IX	do	1,740	4,480	13,600	Do.
June 3	5 days	do	do	1,220	1,180	1,180	Do.
May 29	3 hours	do	Crawford	2,320	0	9,520	Do.
June 3	5 days	do	do	1,060	0	1,880	Do.
May 29	3 hours	do	G. P. 4692	1,920	960	5,040	Do.
June 3	5 days	do	do	700	1,100	1,260	Do.
5	½ hour	1742 X	F. 26	2,840	26,640	119,700	Do.
6	1 day	do	do	2,240	51,760	41,840	Do.
7	2 days	do	do	3,440	900	6,440	Do.
8	3 days	do	do	2,200	1,340	7,280	Do.
9	4 days	do	do	1,360	18,280	20,480	Do.
19	14 days	do	do	4,040	a ∞	∞	Do.
5	½ hour	do	Crawford	8,380	180	28,000	Do.
6	1 day	do	do	5,200	120	8,320	Do.
7	2 days	do	do	7,440	760	13,000	Do.
8	3 days	do	do	3,500	3,160	48,000	Do.
9	4 days	do	do	2,640	1,380	∞	Do.
19	14 days	do	do	1,440	∞	∞	Do.
5	½ hour	do	G. P. 4692	1,380	4,420	4,820	Do.
6	1 day	do	do	3,040	6,320	27,760	Do.
7	2 days	do	do	180	3,420	∞	Do.
8	3 days	do	do	360	2,000	17,960	Do.
9	4 days	do	do	1,880	∞	∞	Do.
19	14 days	do	do	1,740	∞	∞	Do.
May 23	do	1742 VI	do	10,880	3,376	13,180	30 minutes.
23	do	do	Crawford	40,500	2,640	58,800	Do.
23	do	do	F. 26	19,040	18,280	∞	Do.
24	do	1742 VII	G. P. 4692	1,682	0	2,720	Do.
24	do	do	Crawford	1,808	180	7,600	Do.
24	do	do	F. 26	12,040	1,640	26,320	Do.

a This character signifies that there were too many colonies to count.

As is evident from the preceding table the effect of exposure for ten minutes at 54° C., as well as exposure for thirty minutes at the same temperature, is practically in all cases to weaken or suspend the bacteriolytic power of the serum. The few exceptions to be seen in the table are without significance in view of the many cases in which the serum lost in power.

In summing up the results of heat upon the bacteriolytic power of normal hog serum it would seem evident that temperatures below 54° C. are uncertain in action for the lengths of time employed, but that 54° C. even for ten minutes serves quite uniformly to suspend or at least to weaken the bacteriolytic power of the blood serum. A number of tests were made at 55° C. and 56° C., but as these did not show any results that were not to be expected from those at 54° C. it would seem superfluous to give them in detail.

EFFECTS OF INJECTING CULTURES OF B. CHOLERÆ SUIS UPON THE BACTERIOLYTIC POWER OF HOG SERUM FOR THIS ORGANISM.

Very conflicting results have been obtained by various investigators in regard to the effects produced upon the bacteriolytic power of blood serum by the injection of animals with cultures of bacteria. Some have failed to note any effect of such injections, either to in-

crease or diminish the bacteriolytic potency; others have found that the potency was diminished, others again that it was increased. Under the supposition that this discrepancy in results was due perhaps to the tests having been made at different intervals of time after injection, and that the serum taken from the same animal at different periods might exhibit differences in potency, or that it might at certain times be devoid of power, tests were made of serum taken at various intervals after one or more injections. The results of these experiments are summarized in the following tables.

TABLE XVI.—*Bacteriolytic power of serum from the same hog (No. 1754) before and after injection with B. choleræ suis, culture G. P. 4692.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
Crawford	Before injection		
Do	3 days after first injection	2,180	260
Do	9 days after second injection	240	0
Do	10 days after third injection	5,200	0
F. 26	Before injection		
Do	3 days after first injection	16,700	11,800
Do	9 days after second injection	7,600	2,640
Do	10 days after third injection	5,200	26,700
G. P. 4692	Before injection	1,060	3,300
Do	3 days after first injection	638,400	12,800
Do	9 days after second injection	6,340	1,020
Do	10 days after third injection	4,100	16,700

The results given in Table XVI were obtained with the serum from a hog which was injected at various intervals subcutaneously with a beef-broth suspension from an agar culture of the G. P. 4692 strain.

The first injection was made with 5 c. c. of a suspension equivalent in density to a twenty-four-hour typhoid culture diluted about one-half. The second injection was made with 10 c. c. of a suspension of about the same strength. The third injection was made with a suspension consisting of the entire growth in twenty-four hours of an agar culture. Before each injection the blood was drawn from the tail of the animal and placed on ice for three days. After this the serum was drawn off from the blood and tested on the three strains in all cases except the serum from the blood taken at the first drawing, which was tested only upon the culture with which the animal was subsequently injected—culture G. P. 4692.

As there were no tests made with culture Crawford before the animal was injected, it is impossible to be sure whether it was bactericidal for this strain at this time or not, but judging from its action on G. P. 4692 before injection it would seem probable that it would have been bactericidal or at least inhibitory for culture Crawford, since the latter strain was quite uniformly more sensitive to the germicidal action of serum in other experiments. The serum after the injections possessed quite strong bacteriolytic properties for culture Crawford.

It is also possible only to surmise from the results with G. P. 4692 that the serum probably had at least inhibitory power for F. 26 before the animal was injected. It seems to have retained its inhibitory power after the first injections for this organism, but to have lost much in potency after the third injection. With culture G. P. 4692 the results seem to be more or less in accord with those obtained with F. 26, though it is true that three days after the first injection the serum exhibited very strong bactericidal power for G. P. 4692—stronger than for F. 26. Still the results can not be compared too closely in these two cases, since the number of bacteria introduced differed widely.

TABLE XVII.—*Bacteriolytic power of serum from the same hog (No. 1755) before and after injection with B. choleræ suis, culture F. 26.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
Crawford	Before injection		
Do	3 days after first injection	2,180	40
Do	9 days after second injection	240	0
Do	10 days after third injection	5,200	0
F. 26	Before injection	800	47,400
Do	3 days after first injection	16,700	380
Do	9 days after second injection	7,600	11,880
Do	10 days after third injection	8,200	660
G. P. 4692	Before injection		
Do	3 days after first injection	638,400	9,800
Do	9 days after second injection	6,340	3,800
Do	10 days after third injection	4,100	11,200

The results of the experiments with the serum of hog 1755 are recorded in Table XVII. In this experiment the injections were made and the tests applied precisely as with hog 1754, except that culture F. 26 was used for injecting the animal instead of culture G. P. 4692. The concentration and amounts of culture were the same in both experiments, and the methods of procedure otherwise were the same. The serum from the hog before injection was tested upon the F. 26 strain only; after injection it was tested with all three.

For the Crawford culture, as already stated, there was no test of the serum made before the injection of the animal. The tests made with this organism after each of the three injections showed that the serum possessed considerable potency, and there seems no evidence that the injections were followed by diminution of potency for this organism.

For F. 26 culture the serum seems to have had but little potency to start with before injection, but it seems rather to have gained than lost in potency for this organism after injection.

For G. P. 4692 culture the results of the three tests are somewhat difficult of comparison, perhaps, but the serum after the third injection would appear to have been somewhat less potent than that after the second, and, also, after the first, if the difference in the initial doses in these two cases may be disregarded as a factor in determining the results.

TABLE XVIII.—*Bacteriolytic power of serum from the same hog (No. 1756) before and after injection with B. choleræ suis, culture Crawford.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
Crawford	Before injection	700	0
Do	3 days after first injection	400	80
Do	9 days after second injection	240	0
Do	10 days after third injection	5,200	350
F. 26	Before injection		
Do	3 days after first injection	16,700	2,280
Do	9 days after second injection	7,600	25,000
Do	10 days after third injection	8,200	22,700
G. P. 4692	Before injection		
Do	3 days after first injection	638,400	5,960
Do	9 days after second injection	6,340	0
Do	10 days after third injection	4,100	8,300

In the experiment with the serum of the blood of hog 1756, the results of which are given in Table XVIII, the method of procedure was the same as with the serum of hogs 1754 and 1755, just described, the only difference being in the strain of organism employed. In the case of hog 1756 the Crawford culture was used, and the serum was tested upon this organism only before injection and on all three strains after the injections.

With the Crawford culture the serum seems to have been unaffected by the injections of the hog. It seems, at any rate, not to have lost in potency, and from the result after the second injection, if any conclusion is justifiable, it seems possibly to have gained.

With F. 26 it seems to have lost in potency, if the result obtained after the first injection is compared with those after the other two.

With G. P. 4692 culture the serum seems to have lost in potency after the third injection, but it is uncertain whether it gained or lost after the first two.

TABLE XIX.—*Bacteriolytic power of serum from the same hog (No. 1798) before and after injection with B. choleræ suis, culture F. 26.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
F. 26	Before injection	2,620	1,980
Do	10 days after first injection	8,400	7,200
Do	9 days after second injection	5,920	38,000
Do	14 days after third injection	2,400	36,600

In the experiment with the serum of hog 1798, Table XIX, the animal was given three intravenous injections of suspensions in beef broth from agar cultures of the F. 26 strain. The first one was made with a very dilute suspension, weaker than would correspond with a twenty-four hour beef-broth typhoid culture, and the other two injections were made with similar suspensions.

It would appear from the results given in the table that the serum was not actively bactericidal before the injection of the animal, but it seems to have had some inhibitory power. Ten days after the first

injection there seems to have been no striking change. The serum was still inhibitory, but was neither markedly bactericidal nor can it be said to have been devoid of potency. But nine days after the second injection, and fourteen days after the third injection, the serum seems to have lost greatly in the inhibitory power which it possessed. It would appear that in this case the injections had the effect of ultimately diminishing the power of the serum.

TABLE XX.—*Bacteriolytic power of serum from the same hog (No. 1809) before and after injection with B. choleræ suis, culture G. P. 4692.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
F. 26	Before injection	5,920	5,900
Do	14 days after injection	7,300	1,800
Crawford	Before injection	5,700	0
Do	14 days after injection	13,000	56,600
G. P. 4692	Before injection	1,140	10,900
Do	14 days after injection	7,300	7,900

In the experiment given in Table XX the serum of hog 1809 was tested on the three different strains of *B. choleræ suis* before the animal was injected and on the same cultures fourteen days after the hog had received subcutaneously about 5 c. c. of a dilute suspension from an agar culture of culture G. P. 4692. In the tests made with the serum upon the F. 26 strain there seems to have been a slight diminution of potency after the inoculation, but the difference in the numbers of bacteria surviving bacteriolysis in the serum from the hog before injection and those surviving after injection is not large enough to permit of very definite conclusions. The same may be said in regard to the organism with which the hog was injected—culture G. P. 4692—but the results in this case are perhaps somewhat more significant. It would seem justifiable, however, to conclude that there was no marked increase nor decrease of bacteriolytic power for F. 26 culture or for G. P. 4692 produced by the injection. The result with Crawford seems to show plainly that the serum was less potent for this organism after injection than it was before.

TABLE XXI.—*Bacteriolytic power of serum from the same hog (No. 1836) before and after injection with B. choleræ suis, culture F. 26.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
Crawford	Before injection	32,200	*a* 3,840
Do	1 hour after injection	32,200	*a* 5,340
Do	3 days after injection	1,560	*b* ∞
F. 26	Before injection	30,400	*a* 247,800
Do	1 hour after injection	30,400	*b* ∞
Do	3 days after injection	5,000	∞
G. P. 4692	Before injection	35,000	*a* 47,400
Do	1 hour after injection	35,000	*a* 36,600
Do	3 days after injection	6,800	∞

a Serum diluted in the proportion of 1:4.
b This character signifies that there were too many colonies to count.

In the experiment with the serum from the blood of hog 1836, given in Table XXI, the animal was injected intravenously with a suspension from an agar culture of strain F. 26 of about the density of a 24-hour beef-broth culture of the typhoid bacillus. Before injection the hog was bled to obtain serum for testing the power of the normal serum. The amount of blood obtained before injection and that obtained one hour after injection was insufficient for the test to be made with the quantity of serum usually employed, so the serum was diluted in both of these cases with three parts of physiological salt solution to one part of serum. There were no tests made with the undiluted serum.

With the Crawford culture the serum seems to have been quite potent before injection, and also one hour after injection, but three days after injection it appears to have been entirely devoid of bacteriolytic power for this organism.

With F. 26—the strain used to inject the animal—the serum had only weak potency before injection, and if there was any effect produced by the injection it was in the direction of weakening the potency of the serum for this organism. This apparent diminution of potency is to be noted even in one hour after the injection, and is still more pronounced after three days.

With the G. P. 4692 strain the serum was apparently inhibitory at the start before injection and one hour afterwards, but three days after injection it seems to have lost even the power to inhibit the growth of the organism.

The results of this experiment seem to show that in this case the effect of injecting the hog was to suspend the bactericidal power of the serum for the homologous[a] organism as well as for the other strains upon which it was tested.

TABLE XXII.—*Bacteriolytic power of serum from the same hog (No. 1859) before and after injection with B. choleræ suis, culture G. P. 4692.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
Crawford	Before injection	3,760	80
Do	2 hours after injection	3,760	1,460
Do	1 day after injection	600	266,400
Do	2 days after injection	600	18,000
F. 26	Before injection	1,020	2,900
Do	2 hours after injection	1,020	80
Do	1 day after injection	340	39,000
Do	2 days after injection	340	36,400
G. P. 4692	Before injection	2,840	3,800
Do	2 hours after injection	2,840	9,200
Do	1 day after injection	200	54,600
Do	2 days after injection	200	12,400

[a] Homologous is the term now universally employed to denote the organism with which the animal in any given case is injected, as in the present case.

In the experiment given in Table XXII the animal—hog 1859—was injected intravenously with 5 c. c. of a suspension of culture G. P. 4692. The suspension was made in beef broth from a 24-hour agar culture as usual, and was equivalent in density to a 24-hour beef-broth culture of the typhoid culture diluted with about equal amounts of broth. Tests were made of the potency of the serum from the blood of the animal before injection and after injection with all three strains of *B. choleræ suis.* Owing to the fact that the animal was killed in a moribund condition two days after injection, it was impossible to make tests with the serum from the blood later than the second day after injection.

It would seem hardly necessary to discuss the results with the different organisms in detail, since they all appear to point to a lessening of the potency one and two days after injection. Before injection the serum from the blood seems to have been bactericidal for the Crawford strain only; still for the other strains it was at least inhibitory, whereas after injection it had lost much, if not all, even of its inhibitory power on the first and second day after injection. In the tests made two hours after injection the results appear somewhat discrepant; the serum seems to have lost potency for Crawford, and gained for F. 26; but, after all, the figures are perhaps not very significant in these cases.

The results in this experiment may have been complicated by the fact that the animal from which the blood was drawn was suffering from acute infection with *B. choleræ suis* at the time the blood was obtained. It may not be correct to regard this serum as immune in the sense in which this term is usually understood—that is to say, serum from the blood of an animal given injection of bacteria and recovered. Nevertheless, the results are perhaps not without interest, since they show certainly a very marked difference between the serum from the blood of the hog before injection and that from the blood after injection.

TABLE XXIII.—*Bacteriolytic power of serum from the same hog (No. 1860) before and after injection with B. choleræ suis, culture F. 26.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
Crawford	Before injection	1,460	0
Do	1 day after injection	1,240	20
Do	2 days after injection	140	[a] ∞
Do	3 days after injection	5,400	∞
Do	4 days after injection	1,620	0
F. 26	Before injection	2,100	80
Do	1 day after injection	700	880
Do	2 days after injection	1,100	∞
Do	3 days after injection	1,500	∞
Do	4 days after injection	2,260	5,580
G. P. 4692	Before injection	10,200	17,400
Do	1 day after injection	4,100	60
Do	2 days after injection	1,500	∞
Do	3 days after injection	9,900	∞
Do	4 days after injection	1,380	1,020

[a] This character signifies that there were too many colonies to count.

In the experiment recorded in Table XXIII, the animal—hog 1860—was injected first with about 2 c. c. of a dilute suspension of an agar culture in beef broth, but most of the material was injected merely under the skin, as the attempt to strike a vein was not successful. Two days after the first injection a second injection of about 2.5 c. c. of a similar suspension from the same strain of bacterium, in this case all of the suspension, was introduced into the vein. Two days after the second injection a third injection was made similar in all respects to the second. The suspensions for all three of the injections were heated at 57° to 58° C. for thirty minutes. The blood was drawn from the animal before injection and on the first, second, third, and fourth days after the last injection. The results seem to be quite uniform for the serum on the second and third days after the injections; on both days the serum showed apparently entire loss of bactericidal power for all three organisms. On the fourth day after the last injection the serum had apparently gained in potency, particularly for the Crawford strain. The results obtained with the serum one day after the last injection are not very striking, but they perhaps permit of the conclusion that the serum had not lost in potency at least to any marked extent.

As previously stated, the results of the injection of animals with cultures or with the products of growth of bacteria have been most conflicting in various hands in so far as the effect of such injections upon the bacteriolytic properties of the blood serum from the animal in test-tube experiments is concerned.[7]

A number of investigators have found that the injection of an animal with cultures or with the products of growth of certain bacteria increases the bactericidal power of the blood serum for the homologous organism. Others have found that there is no effect upon the bactericidal potency of the serum produced by such injections, while still others have found diminution or complete loss of power for the homologous organism to follow the injections. Among others Neisser and Wechsberg[14] have found that the so-called immune serum—i. e., the serum from an animal which has received injections of certain bacteria—no longer possesses bactericidal power for the homologous organism when the serum is undiluted. The results with the diluted serum will be discussed later. Buxton[4] has found that the serum from the blood of a normal rabbit is capable of destroying very large numbers of the typhoid and paratyphoid bacilli, but that after the rabbit has been injected with these organisms it no longer kills any of the homologous strain.

But while there is undoubtedly conflict in regard to the matter, the preponderance of evidence certainly seems to show that the serum is frequently, if not always, weakened in bactericidal power or completely robbed of this power for the homologous organism by these

injections, and the results recorded above seem in many instances to bear out this result in the case of hogs injected with *B. choleræ suis*. But it would also seem apparent that there is a tendency in some cases to a restoration of the lost potency on further injection of the animal.

These conclusions seem justifiable at least from the experiments with the F. 26 and the G. P. 4692 strains. The results with the Crawford strain differ somewhat in this respect from those with the other two strains, and this may be due to the fact, already mentioned, that this organism is of only feeble pathogenic potency. It seemed to be much more easily killed by the serum generally than the other two strains, and it probably also has less power than these to cause the reaction in the serum which is necessary for the production of immune serum as this is understood. The serum of hogs injected with this strain would consequently perhaps be expected to behave more like the serum from an uninoculated animal. In some cases the serum of the blood of hogs injected with the F. 26 or with the G. P. 4692 strain seemed to have retained bactericidal power for the Crawford strain after having lost it for the other two.

The variations which have been already alluded to as occurring in the serum of animals from time to time, and which are due to causes as yet obscure, must be borne in mind in drawing conclusions from the results of experiments with the serum from animals injected with cultures. Of course it is impossible to determine in the present case the extent to which this spontaneous variation, as it is at present convenient to call it, may have affected the results.

In view of the many conflicting results with the serum of the blood both of normal and of injected animals, in so far as the bactericidal properties are concerned, it is evident that there are some obscure factors in the process which the methods at present in use are incapable of determining. If the blood serum from one and the same animal is found to be bactericidal at one time and not at another, where the animal is kept under apparently the same conditions, and if, furthermore, the injection of cultures is found by certain observers to have no effect upon the bactericidal potency of the serum, by others to increase the potency, and by others again to lessen the potency, there must be some very subtle factors which enter into the problem, else they would scarcely be overlooked by so many investigators.

EFFECTS OF DILUTION UPON THE BACTERIOLYTIC POWER OF NORMAL AND IMMUNE SERUM.

DILUTION OF NORMAL SERUM.

The effects of dilution upon the bactericidal power of hog serum were tested in the manner usually employed in such cases. The method for obtaining the blood was to cut off a piece of the tail and allow the blood to run into sterile test tubes or flasks, which were put into the

refrigerator immediately. The serum was drawn off as soon as it had separated, and 1 c.c. of it was put into each of three tubes. Various dilutions, as mentioned in the tables, were also prepared with physiological salt solution, and portions of these dilutions were distributed into test tubes, three tubes from each dilution, each containing 1 c.c. In most cases 0.1 or 0.05 c.c. of neutral, peptonized beef broth was added to insure sufficient nutrient material for the bacteria which were subsequently introduced. This was done in conformity with the method ordinarily employed in such experiments. Each of the tubes was finally inoculated with a carefully measured amount, usually 0.05 c.c., of a suspension in physiological salt solution from a recent agar culture. One series each was inoculated with one of the three strains of *B. choleræ suis*. In addition to the tubes of undiluted and of diluted serum, three tubes of physiological salt solution with the addition of 0.05 c.c. of beef broth were also prepared in each case, and inoculated at the same time as the serum tubes with an amount of culture equal to that used for the serum tubes. These tubes constituted the checks. Immediately after the inoculation of the tubes, agar plates were made from each of the tubes of salt solution with carefully measured amounts, and after twenty-four hours at room temperature agar plates were made from all the tubes of a series—from both the undiluted and diluted serum, and from the salt solution. In nearly all cases 0.05 c. c. was used for each plate. The colonies were counted after twenty-four or forty-eight hours in the incubator at body temperature.

TABLE XXIV.—*Bacteriolytic power of normal hog serum on dilution.*

No. of hog.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 50.	Diluted 1 to 500.	Diluted 1 to 5,000.	
1798	Crawford	840	0	26,400	81,200	140	.....
	F. 26	2,620	1,980	1,400	1,740	2,280	.....
	G. P. 4692	2,660	1,680	2,700	32,800	5,200	.....
1808	Crawford	840	0	0	74,200	2,340	.....
	F. 26	8,400	2,040	35,200	[a]∞	161,000	.....
	G. P. 4692	2,060	2,900	2,980	1,980	900	.....
1809	Crawford	5,700	0	520	∞	∞	∞
	F. 26	5,920	5,900	109,200	∞	∞	∞
	G. P. 4692	1,140	10,900	2,880	123,200	60,800	73,000
				Diluted 1 to 10.	Diluted 1 to 100.	Diluted 1 to 1,000.	
1836	Crawford	32,200	[b]3,840	320	135,800	∞	∞
	F. 26	30,400	[b]247,800	117,600	∞	∞	∞
	G. P. 4692	35,000	[b]47,400	37,200	308,000	∞	∞
1859	Crawford	3,760	80	1,160	149,800	∞	∞
	F. 26	1,020	2,900	13,000	187,600	∞	∞
	G. P. 4692	2,840	3,800	6,200	16,080	∞	∞
1860	Crawford	1,460	0	80	12,500	118,400	18,700
	F. 26	2,100	80	30,480	504,600	879,200	∞
	G. P. 4692	10,200	17,400	11,400	61,400	445,200	206,000

[a] This character signifies that there were too many colonies to count. [b] Diluted 1 to 4.

The results given in Table XXIV show that the serum taken from a hog before injection—i. e., normal serum—as a rule, loses in potency upon dilution; that is to say, a given amount of the diluted serum kills actually fewer bacteria than the same amount of the undiluted serum. This was to be expected a priori and from the results of others with other organisms and with other animals. But the point which seems specially worthy of note, and which will be discussed in a different connection in this paper, is that the diminution of potency on dilution is not in proportion to the degree of dilution. Not only is this the case, but in at least 4 out of the 18 experiments given in the table there was apparently an actual increase of power on dilution. Those familiar with the subject will at once see the bearing of these results upon the difference between the bacteriolytic properties of normal serum upon the one hand and of immune serum on the other. As it is attempted to show below, the difference seems, after all, to be one of degree only, and not a difference of kind.

In interpreting the above results it must of course be borne in mind that the number of bacteria found in the serum after twenty-four hours probably does not represent merely the bacteria which are not killed by the serum, but it represents in addition the number resulting from the multiplication of these after bacteriolysis has ceased. Thus, in the case of the serum from hog 1859, if 1 c. c. killed approximately 4,000 of the Crawford bacilli, 0.1 c. c. of the serum should evidently have killed 400 and have left 3,600 to multiply, unless in addition to the bactericidal power the serum also possessed inhibitory power for the bacteria which survived bacteriolysis. If the serum had exhausted its bacteriolytic power in ten or twelve hours and exerted no further influence after this time, there should have been in the 1 to 10 dilution after twenty-four hours a multiplication of the 3,600 bacilli left, resulting in some 4,000,000 or 5,000,000 even at the rate of only one generation an hour, as a simple calculation shows. But even if only one bacillus were left after twelve hours, there would result over 4,000 in twenty-four hours, provided there were no hindrance to multiplication at the rate assumed above. It would seem, therefore, that either relatively more bacilli were destroyed by the diluted serum than by the undiluted, or that, aside from the bacteriolytic action, the diluted serum exerted an inhibitory effect in the experiment quoted; for at the end of twenty-four hours there were only about 1,000 bacilli in the 1 to 10 dilution instead of the 4,000 calculated as the minimum number possible under the supposition stated.

The dilution of 1 to 1,000 in the same experiment should evidently have killed only 40 bacilli if it acted with the same relative intensity as the undiluted, i. e., if it possessed just one one-thousandth of the potency of the undiluted serum. Supposing that all bacteriolytic action ceased after fourteen hours, the 3,960 bacilli left alive increasing at the rate of one generation an hour would be represented by over 4,000,000

bacilli in ten hours, whereas as a matter of fact there were only about 32,000 in this dilution after twenty-four hours.

The time at which bacteriolysis is exhausted in serum at room temperatures was not determined, but tests made at the end of three or four hours showed that up to this time there was little or no appreciable effect upon the number of bacteria introduced, so that the above assumption that the process is exhausted in twelve or fourteen hours may or may not be correct. But whether it is or is not, the relative effect of the various dilutions would be shown by assuming the process to come to an end simultaneously—and this is probably the case—at any time within the twenty-four hours, the object of the calculation being merely to show that no definite rule of proportion could be observed, and that the diluted serum in all cases destroyed relatively more bacteria than the undiluted serum. While dilution of normal serum causes a marked diminution in the bactericidal power, it does not seem to diminish it to the extent that would be expected a priori. In other words, a dilution of 1 to 10 does not seem to destroy just one-tenth the number of bacilli that the undiluted serum destroys, but a much larger number than this, or at least if it does not destroy a larger number it prevents as relatively great an increase as that which takes place in the undiluted serum.

Still another method of comparison which appears permissible, and which leads to the same conclusion, is to calculate the number of bacteria which should result in a dilution of 1 to 100 if the same relative increase took place as in the 1 to 10 dilution; or to compare the relative increase in any of the dilutions with that in the undiluted serum in the experiment given above. For such a comparison it may be assumed that the organisms which were not destroyed in fourteen hours multiplied in the undiluted and in the diluted serum at the same rate, or at least that if the serum retarded multiplication this effect was more marked in the more concentrated serum than in the more dilute. It would seem evident, then, that the relatively smaller number of bacteria in the diluted serum can be accounted for only on the supposition that the diluted serum retains more bacteriolytic power than would be expected from the degree of potency shown by the undiluted serum, according to the simple rule of proportion. This point seems not to have been alluded to in the literature, but it would seem nevertheless not without significance perhaps, since it tends apparently to show that the difference between normal serum on the one hand and immune serum on the other is merely a difference of degree and not a difference of kind. The matter of the actual identity in the behavior of normal sera and of immune sera on dilution, in spite of the apparent difference shown by them, will be discussed more fully in a different connection below, where the attempt is made to show that the law of complement diversion applies to normal serum as well as to immune serum.

DILUTION OF IMMUNE SERUM.

The results obtained by tests made to determine the effect of dilution upon the bacteriolytic power of immune hog serum—that is to say, of serum from hogs injected with cultures—in the main agree with those observed in all recent investigations in this direction with the serum of other animals injected with typhoid, paratyphoid, Asiatic cholera, and other bacteria. The behavior of the serum under such conditions is very peculiar; it seems to be in conflict with all laws of proportion which are applicable to other chemical reagents, particularly in regard to germicidal substances in general. With germicidal substances in general, as it is hardly necessary to state, the more concentrated the germicide the more powerful the destructive power. A strong solution of carbolic acid is a more powerful germicide than a weaker solution. But not so with immune blood serum. Paradoxical as it may seem, the bacteriolytic potency of the immune serum increases upon dilution. The undiluted immune serum may be weak or wanting in bactericidal properties, and yet become more and more potent upon dilution. In other words, 1 c. c. of undiluted immune serum may be found to kill few, if any, bacteria; it may be found even to furnish a good nutrient medium in which the bacteria grow; whereas 1 c. c. of a dilution consisting of 1 part of the same serum to 10 or 100 or 1,000 parts of dilute salt solution may be found to kill many hundred bacteria introduced into it. It goes without saying, of course, that there is a limit to this increase of potency upon dilution. Where the dilution goes beyond a certain point the potency becomes weakened. There is for each immune serum an optimum dilution for bacteriolysis, above and below which there is less and less potency. Thus, as will be seen in some of the experiments described below, the greatest potency in one case was in a dilution of 1 part of serum to 100 parts of salt solution. In the case of a different serum the optimum dilution for bacteriolysis was 1 part of serum to 1,000 parts of salt solution.

Buxton very appropriately speaks of the phenomenon just described as the formation of bacteriolytic zones, of which three may be recognized in every immune serum—a pro-killing zone, a killing zone, and a post-killing zone. The action of the undiluted immune serum lies in the pro-killing zone, that of the optimum dilution lies in the killing zone; that of dilutions in which bacteriolysis becomes again weakened lies in the post-killing zone. Of course it is to be understood that these zones are not sharply marked off from one another, but that they gradually merge into each other. The pro-killing zone, for example, may extend over dilutions of several strengths, and the same is true of the killing and of the post-killing zones. Thus in some of the experiments given below there appeared to be no marked difference between the bactericidal power of the undiluted serum and the same serum diluted

in the proportion of 1 to 10 or 1 to 100; the pro-killing zone in these cases would be said to extend to the dilutions of 1 to 10 or 1 to 100. The zones are therefore spoken of as longer or shorter, according to whether they apply to many or to few dilutions.

The peculiar behavior of immune serum just described was first observed by Neisser and Wechsberg,[14] and is called the Neisser-Wechsberg phenomenon. While it is true that this phenomenon was observed quite frequently in immune hog serum, as will be shown later, it was not as noticeable in some cases as it was in others, and moreover the three zones varied in length, not only after the injection of different strains of *B. choleræ suis* in different hogs, but also after the injection of the same strain in different hogs.

It has already been shown that normal serum of hogs gains relatively in potency on dilution, and, as has just been stated, immune serum also gains in potency. The two behave alike, and at most there is only a difference of degree between the amount of gain in either case. But even a difference of degree between the two sorts of serum is not always noticed in the present experiments, for the normal serum acted in some cases just like immune serum. It was not only relatively more potent on dilution, but it killed actually more bacteria per 1 c. c. of serum in certain dilutions than in dilutions containing more of the serum.

TABLE XXV.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1859, injected with culture G. P. 4692.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 10.	Diluted 1 to 100.	Diluted 1 to 1,000.	
Crawford..	Before injection.....	3,760	80	1,160	149,800	[a]∞	∞
	2 hours after injection..	3,760	1,460	200	4,900	∞	∞
	1 day after injection....	600	266,400	4,500	80	520	1,580?
	2 days after injection...	600	18,000	700	4,500	12,400	1,580?
F. 26.....	Before injection.....	1,020	2,900	13,000	187,600	∞	∞
	2 hours after injection..	1,020	80	11,400	162,400	∞	∞
	1 day after injection....	340	39,000	27,800	480	5,500	2,700?
	2 days after injection...	340	36,400	1,800	9,440	900	2,700?
G. P. 4692..	Before injection.....	2,840	3,800	6,200	16,080	∞	∞
	2 hours after injection..	2,840	9,200	27,400	15,200	∞	∞
	1 day after injection....	200	54,600	22,400	19,200	60	200?
	2 days after injection...	200	12,400	140	400	1,600	200?

[a] This character signifies that there were too many colonies to count.

An example of the difference often to be seen between the bactericidal power of the serum from a hog before and after injection with cultures of *B. choleræ suis*, in so far as the effect of dilution is concerned, is given in Table XXV. The animal in this case was injected with a culture of the G. P. 4692 strain, and the blood was drawn before and at several intervals after injection. Before the injection the serum from the blood of this hog showed inhibitory, but no bactericidal power for the organism with which the animal was injected.

In this case there were 2,840 of these organisms introduced per 1 c. c. into the undiluted serum, and after twenty-four hours there were 3,800 per 1 c. c. present. In the dilution of 1 to 10 the same number of organisms introduced about doubled in number in twenty-four hours; in the dilution of 1 to 100 the same number of organisms increased about fivefold; in the 1 to 1,000 dilution there was a countless increase. In the undiluted serum from the blood of the same hog two days after inoculation there was a sixty-two fold increase of the 200 bacilli per 1 c. c. which were introduced, while in the 1 to 10 dilution with the same number of bacilli introduced there were only 140 alive after twenty-four hours, in the 1 to 100 dilution only 400, and in the 1 to 1,000 there were 1,600. In other words, the normal serum diminished in potency on dilution while the immune serum increased in potency on dilution. It should be noticed, however, that the diminution of potency of the normal serum is an actual and not a relative decrease on dilution. In this case, as in others, the normal serum killed more bacteria, relatively, when it was diluted than it did when undiluted.

TABLE XXVI.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1798, injected with culture F. 26.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 50.	Diluted 1 to 500.	Diluted 1 to 5,000.	
F. 26.....	Before injection.....	2,620	1,980	1,400	1,740	2,280	.....
	10 days after first injection.	8,400	7,200	106,400	103,600	6,900	.....
	9 days after second injection.	5,920	38,000	38,400	84,000	[a]∞	∞
	14 days after third injection.	2,400	36,600	[b]86,800	[b]81,200	[b]112,000	∞
Crawford..	10 days after first injection.	840	0	26,400	81,200	140	.....
	9 days after second injection.	5,700	160	400	27,600	∞	∞
	14 days after third injection.	13,000	359,600	[b]12,000	[b]20,400	[b]175,000	∞
G. P. 4692..	10 days after first injection.	2,660	1,680	2,700	32,800	5,200	.....
	9 days after second injection.	1,140	1,600	2,000	5,500	26,600	73,000
	14 days after third injection.	7,300	5,100	[b]9,100	[b]7,500	[b]∞	∞

[a] This character signifies that there were too many colonies to count.
[b] Dilutions used were 1 to 10, 1 to 100, 1 to 1,000.

That there is a difference in the behavior of the blood serum of different hogs injected with the same strain of *B. choleræ suis* is shown by comparing the results given in Table XXVI, hog 1798, with those shown in Table XXVII, hog 1860. Both of the animals were injected with culture F. 26. Before injection the blood of hog 1798 showed considerably more bacteriolytic power on dilution for the organism with which the animal was afterwards injected than the blood serum of hogs did in other cases, though, as has been seen, normal hog

serum frequently retains a considerable degree of potency upon dilution. This serum appears to have possessed about an equal degree of potency in all the dilutions which were tried, and to have been about as potent when diluted as it was when undiluted. Ten days after injection its behavior was very peculiar, for it was apparently about as potent as the normal serum when it was undiluted and in the 1 to 5,000 dilution, but it had lost potency in the dilutions of 1 to 50 and 1 to 500. Nine days after a second injection it had apparently lost in bactericidal potency, both when diluted and undiluted. Fourteen days after a third injection it seems to have gained in power in all strengths. If this is compared with the behavior of the serum from the blood of hog 1860, Table XXVII, a hog also injected with the F. 26 strain, it will be noticed that there is a marked difference.

TABLE XXVII.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1860, injected intravenously on four successive days with culture F. 26, about 5 c. c. heated for thirty minutes at 58–59° C.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 10.	Diluted 1 to 100.	Diluted 1 to 1,000.	
Crawford..	Before injection.....	1,460	0	80	12,500	118,400	18,700
	1 day after injection....	1,240	20	14,800	2,220	5,200	5,760
	2 days after injection...	140	[a]∞	∞	19,000	6,600	460
	3 days after injection...	5,400	∞	[b]∞	[b]∞	[b]∞	∞
	4 days after injection...	1,620	0	0	2,300	14,400	20,400
F. 26.....	Before injection.....	2,100	80	30,480	504,600	879,200	∞
	1 day after injection....	700	880	2,160	70,000	98,000	10,000
	2 days after injection...	1,100	∞	∞	200,200	156,800	64,400
	3 days after injection...	1,500	∞	[b]∞	[b]∞	[b]∞	∞
	4 days after injection...	2,260	5,580	1,820	2,860	5,900	13,600
G. P. 4692..	Before injection.....	10,200	17,400	11,400	61,400	445,200	206,000
	1 day after injection....	4,100	60	8,800	10,600	98,000	28,000
	2 days after injection...	1,500	∞	∞	64,400	46,800	4,400
	3 days after injection...	9,900	∞	[b]∞	[b]∞	[b]∞	∞
	4 days after injection...	1,380	1,020	2,100	2,480	10,800	∞

[a] This character signifies that there were too many colonies to count.
[b] Dilutions used were 1 to 50, 1 to 500, 1 to 5,000.

In the experiment with the blood serum of hog 1860, Table XXVII, the animal was injected on four successive days with quite dense suspensions, considerably denser than a twenty-four-hour culture of typhoid. The suspensions were heated for thirty minutes at 58–59° C. before injection, and the injections were made intravenously. On each day before injection, and previous to any injection, blood was drawn from the tail of the animal, and after the separation of the serum this was used on the three strains of *B. choleræ suis*. Doubtless the fact that the tests were made while the animal was being immunized had some effect upon the results obtained, and for this reason they are different in some measure from those obtained in other experiments in which the serum was tested only after the animal had been immunized.

The undiluted serum from this hog before injection was quite strongly bactericidal for the Crawford and the F. 26 strains, but it

was at most merely inhibitory for the G. P. 4692 strain. After the second and third injections the serum seems to have lost all potency for all three organisms, at least when undiluted. But after the fourth injection the serum seems to have regained its potency both when undiluted and in certain of the dilutions.

TABLE XXVIII.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1835, injected with Crawford culture.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 10.	Diluted 1 to 100.	Diluted 1 to 1,000.	
Crawford..	1 hour after injection ..	5,860	18,200	0	33,600	[a] ∞	∞
	1 day after injection....	3,200	71,100	5,200	11,000	∞	∞
F. 26.....	1 hour after injection ..	16,600	∞	110,400	∞	∞	102,200
	1 day after injection....	6,900	0	40,400	∞	∞	∞
G. P. 4692..	1 hour after injection ..	9,600	31,000	7,200	28,000	∞	∞
	1 day after injection....	4,600	3,600	96,000	10,700	∞	1,280?

[a] This character signifies that there were too many colonies to count.

In the experiment of which Table XXVIII gives the summary the hog was injected intravenously with a dilute suspension of the Crawford culture. Two injections of the organism were given on two consecutive days, and blood was drawn from the animal one hour and twenty-four hours after the last injection. No blood was drawn before injection in this case.

There seems to have been no difference between the behavior of the blood on dilution and that of ordinary normal blood on dilution. Omitting one or two unimportant discrepancies, the serum lost more and more in potency upon further and further dilution.

TABLE XXIX.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1809, injected with culture G. P. 4692.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 50.	Diluted 1 to 500.	Diluted 1 to 5,000.	
F. 26.....	Before injection.....	5,920	5,900	109,200	[a] ∞	∞	∞
	14 days after injection..	2,400	1,800	[b] 168,000	[b] 224,000	[b] ∞	∞
Crawford..	Before injection.....	5,700	0	520	∞	∞	∞
	14 days after injection..	13,000	56,600	[b] 4,000	[b] 6,600	[b] ∞	∞
G. P. 4692 ..	Before injection.....	1,140	10,900	2,880	123,200	60,800	73,000
	14 days after injection..	7,300	7,900	[b] 9,200	[b] 25,900	[b] ∞	∞

[a] This character signifies that there were too many colonies to count.
[b] Diluted in the proportion of 1 to 10, 1 to 100, 1 to 1,000.

In the experiment summarized in Table XXIX the hog was injected subcutaneously with 5 c. c. of a suspension of G. P. 4692, but there seems to have been little if any effect produced upon the bacteriolytic power of the serum. It is true that with the Crawford strain there is some evidence of loss of potency in the undiluted serum, and of increased potency upon dilution in the serum after the injection of the animal, but the effects on the whole are not striking.

TABLE XXX.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1801, injected with Crawford culture.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c.c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 50.	Diluted 1 to 500.	Diluted 1 to 5,000.	
F. 26.....	10 days after first injection.....	8,400	660	24,400	[a]∞	172,000	.....
	9 days after second injection...	5,900	4,160	19,800	∞	∞	∞
	14 days after third injection....	2,400	60	[b]99,400	[b]196,000	[b]∞	∞
Crawford..	10 days after first injection.....	840	0	640	7,000	1,460	.....
	9 days after second injection...	5,700	120	19,000	249,200	∞	∞
	14 days after third injection....	13,000	6,220	[b]8,400	[b]12,500	[b]6,400	∞
G. P. 4692..	10 days after first injection.....	2,060	3,500	2,540	3,820	2,120	.....
	9 days after second injection...	1,140	2,020	2,880	3,320	7,600	73,000
	14 days after third injection....	7,300	7,800	[b]11,300	[b]8,100	[b]21,000	∞

[a] This character signifies that there were too many colonies to count.
[b] Diluted 1 to 10, 1 to 100, 1 to 1,000.

The hog (1801) in the experiment shown in Table XXX was injected on three different occasions with the Crawford organism. No tests were made of the serum before the inoculation of the animal, but there seems little or no evidence of the reaction usually seen in the serum of animals injected with bacteria in the tests which were made after each injection. The serum in most of the tests showed loss of bacteriolytic power on dilution, behaving in this respect like normal serum. It is true that with the homologous strain—Crawford—the serum ten days after the first injection and fourteen days after the third injection appeared to be somewhat more strongly bacteriolytic in the dilution of 1 to 5,000 and 1 to 1,000, respectively, than in the less diluted serum; but on the whole the serum seemed to act more like normal than like immune serum.

TABLE XXXI.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1836, injected with culture F. 26.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 10.	Diluted 1 to 100.	Diluted 1 to 1,000.	
Crawford..	Before injection.....	32,200	[a]3,840	320	135,800	[b]∞	∞
	1 hour after injection ..	32,200	[a]5,340	240	∞	∞	∞
	3 days after injection...	1,560	∞	0	151,400	25,200	42,000
F. 26.....	Before injection.....	30,400	[a]247,800	117,600	∞	∞	∞
	1 hour after injection ..	30,400	[a]∞	∞	∞	∞	∞
	3 days after injection...	5,000	∞	98,000	∞	∞	∞
G. P. 4692..	Before injection.....	35,000	[a]47,400	37,200	308,000	∞	∞
	1 hour after injection ..	35,000	[a]36,600	8,400	109,200	∞	∞
	3 days after injection...	6,800	∞	13,800	75,600	285,600	553,600

[a] Diluted in the proportion of 1 part of serum to 3 of physiological salt solution.
[b] This character signifies that there were too many colonies to count.

In the experiment with the serum from the blood of hog 1836, Table XXXI, the animal was given intravenously 5 c.c. of a dilute beef-broth suspension of an agar culture of F. 26 strain. One day after the first

injection the animal was given a second injection of the same amount of the same strain. The blood was drawn before any injection was made, one hour after the first injection, and three days after the second injection.

The results obtained with the serum from the blood of this hog, at least in so far as the strains G. P. 4692 and Crawford are concerned, are in striking contrast with the results obtained with the serum from the blood of hog 1801 (Table XXX). In the case of hog 1836 the animal was injected with a more virulent strain than was the case with hog 1836, and it is possible that this may have had some influence upon the results in the two cases. But aside from this the two sera must have had different potency originally, for even the normal serum of the blood of hog 1836, before the animal was injected, will be seen to have shown increase of potency upon dilution; the dilution of 1 to 10 was more potent than the dilution of 1 to 4. There was no test made of the undiluted normal serum for the reason that not enough of the blood was obtained to use for the purpose. This increase of potency on dilution of the normal serum of hog 1836 is to be seen particularly in the tests with the Crawford strain, in which the dilution of 1 to 4 showed 3,840 bacilli per 1 c. c. whereas the dilution of 1 to 10 showed only 320 bacilli after the same exposure—one day in each case—with the same number of organisms—32,200 per 1 c. c.—introduced. This serum in other words seems to have behaved somewhat like immune serum to start with, and this behavior seems to have been intensified by the injections, as will be seen by examining the table.

SUMMARY OF THE DILUTION EXPERIMENTS.

On summing up the results of the experiments made with normal and immune hog serum on dilution, it will be noticed that the normal serum seems to differ greatly in its behavior in different cases; that while it sometimes shows marked diminution of potency upon dilution it sometimes shows increased potency on dilution. The immune hog serum also shows variation in behavior.

But the results which would seem to be of especial interest are those in which the immune serum showed strong bacteriolytic power when undiluted, and lost in power on dilution in certain proportions, but gained potency upon further dilution. In these cases there appeared to be a combination of the behavior of normal and of immune serum, as this is usually stated to occur.

As already stated above, the characteristic behavior of immune serum is said to consist of the formation of bacteriolytic zones. A typical immune serum reaction consists of a pro-killing zone, a killing zone, and a post-killing zone on dilution. In the pro-killing zone, represented by the undiluted immune serum, there is no bacteriolysis. In the killing zone, represented by one or more dilutions, there is

bacteriolysis. In the post-killing zone, represented by further dilution than the killing zone, there is again lack of bacteriolysis. But in the results at present under consideration the serum showed a killing zone to begin within the undiluted serum, thus imitating to this extent the normal serum.

The peculiar behavior just alluded to was to be seen in several instances given in the preceding tables. Perhaps the most striking examples are furnished by the behavior of the immune sera from hogs 1798 and 1808 (see Table XXIV). In these sera with the Crawford culture there was strong bacteriolysis shown in both cases undiluted. The 1808 was still strongly potent in the 1 to 50 dilution, but the 1798 serum was much less potent in this dilution than when undiluted. Both sera seem to have lost all potency when diluted in the proportion of 1 to 500. But the point to be specially noted is that they both seem to have gained greatly in the dilution of 1 to 5,000; particularly is this the case with the 1798 serum.

For the present it is merely necessary to call attention to these results; they will be taken up and discussed in another connection further along in the present paper.

GENERAL DISCUSSION.

Judging from the results of the above experiments as a whole, it seems apparent that the blood serum of hogs has but feeble bactericidal potency for *B. choleræ suis*, at least for the more strongly pathogenic strains. This would naturally lead to the presumption that the known susceptibility of hogs to infection by intravenous injection was due to this cause, but it does not seem as yet settled that the susceptibility of an animal in general is in direct proportion to the want of bactericidal power of its blood. In fact, in some instances, as is well known, quite the contrary has been shown to be the case. Nuttall, Buchner, and many others have shown that rabbit serum in test tubes will kill large numbers of anthrax bacilli, and yet a comparatively small number of these organisms introduced beneath the skin of a rabbit is certainly fatal to the animal. Guinea-pig serum, as recently shown by Buxton,[4] may be expected to kill approximately 1,000,000 typhoid bacilli per 1 c. c., and yet the injection of a relatively small amount of this organism intraperitoneally is certainly fatal for guinea pigs. In spite of this, however, as Wolff[20] has pointed out, it is probably all a matter of dosage, and bacteriolysis is an efficient means of defense where the number of bacteria concerned is not large. On the contrary, where the number of bacteria exceeds certain limits, bacteriolysis is not only not a means of defense, but it actually liberates the disease-producing substances—the endotoxins—from the bodies of the bacteria.

Pfeiffer[17] was the first to give this name, endotoxins, to the poisonous substances which are present in the bodies of the bacteria of certain species, and it is now quite generally the opinion that it is the liberation of these that brings about disease in infection with these bacteria. So long as bacteria of this kind remain intact, they are thought by those holding this view to be incapable of producing disease. Mechanical injury and other harmful effects formerly attributed to the action of bacteria are now thought by many not to exist.

The effects of inoculations of hogs with cultures of *B. choleræ suis*, and the conclusions to be drawn from these experiments are on the whole in harmony with those derived from experiments with various bacteria injected into rabbits, guinea pigs, and other animals. Thus Loeffler and Abel[12] found that undiluted immune blood serum from dogs failed to protect guinea pigs from injections of virulent colon bacilli. Neisser and Wechsberg[14] obtained analogous results with immune rabbit serum in test tubes, the undiluted immune serum failing to destroy the bacteria of the kind used to produce the immune serum. Buxton,[4] as already quoted, found that the undiluted serum of rabbits injected with cultures of typhoid or paratyphoid bacilli behaved in a similar manner. It was to be expected, therefore, that the serum of hogs injected with cultures of *B. choleræ suis* would show loss of bacteriolytic power in undiluted serum. The behavior of the diluted serum will be considered below.

In the foregoing experiments with the serum from the blood of hogs injected with cultures of *B. choleræ suis*, it appears that the effect of the first injection was sometimes, as stated, to deprive the undiluted serum of its power to kill *B. choleræ suis*. It is true that this behavior was frequently lacking. But if the results after several injections are examined, it will be found that the undiluted serum frequently regains its power. This is noticeable in a number of cases if not in all, and it perhaps accounts for the experience of so many observers that animals frequently die from injections of comparatively small doses after having been treated with carefully graded amounts of culture. Thus Wolff[20] found that strong, healthy rabbits, instead of becoming more and more accustomed to injections of cultures, withstood these less and less readily, even without increasing the dose; that after remaining apparently unaffected by previous injections, they often die suddenly on the inoculation being repeated with the same sized dose. This supersensitiveness on repeated injections has often been observed in various animals and with various bacteria. It has also been met with in the course of the present experiments with hogs. In fact, it does not seem so difficult to grade the first dose so as to avoid killing the animal as it is to strike upon the proper subsequent doses. The return of bacteriolytic properties in the serum following further injection of animals may account for

this result, the first injections depriving the serum of the bacteriolytic power and thus preventing the liberation of endotoxin and its consequences, and the subsequent injections producing a return of bacteriolytic power and causing the liberation of the endotoxin.

It would seem perhaps difficult to reconcile the fact that the serum becomes more potent for the protection of animals other than the individual which produces it, and that yet from the very fact of its bacteriolytic power it should be injurious to the animal furnishing it. It would appear paradoxical to find that serum obtained from an animal possessed the property of protecting other animals, and, at the same time, not only failing to protect the animal furnishing it, but actually increasing the susceptibility of this animal. Here again Wolff offers the explanation that it is a matter of dosage. If the number of bacteria injected into the animal furnishing the serum, or into the animal which has been given a protective dose of the serum, is not so large that on being disintegrated the amount of toxin is sufficiently large to cause disease, the animal escapes. There are apparent difficulties in the way of this explanation, but it is, perhaps, nevertheless not without weight.

In this connection the results obtained by Elischer and Kentzler[6] on comparing the bactericidal power of unheated immune serum—i. e., immune serum containing its own proper complement—with the same serum heated and reactivated by the addition of fresh rabbit serum would seem to apply. The latter was found to be more potent than the former, and this would seem in a way to offer an explanation of the protective power of immune serum for an animal into which it is injected on the one hand and of the failure of such protective properties of the same serum for the animal from which it was obtained on the other. Elischer and Kentzler interpret their results as indicating that immune serum contains an insufficient amount of complement for the full development of its bactericidal properties. So in other cases, the amount of complement in immune serum may be insufficient to enable the serum to protect the animal from which it was obtained, while it would find sufficient complement in the serum of a fresh animal. To state this view briefly, the excess of amboceptors in immune serum finds the requisite amount of complement in the normal serum of uninfected animals.

When the behavior of hog serum on dilution is considered, the explanation afforded by the theory of complement diversion seems applicable, not only for the behavoir of immune serum in which there was shown often to be increased bactericidal potency on dilution, but also in the case of normal serum, as it will be attempted to show.

It will be recalled from the statements in regard to the theory of complement diversion given on page 13 that in accordance with the

views of the Ehrlich school generally it is held by Neisser and Wechsberg, the originators of the theory, that bacteriolysis is due to the action of a substance called complement, which is present in normal and in immune serum, but that this substance can act on the bacteria only when it becomes united to these through the medium of certain bodies called amboceptors. The complements are held to be incapable of uniting directly with the bacteria. Now, according to the theory of complement diverson, if there are more amboceptors than complements present in any given serum, all the complements which are present unite with a corresponding number of amboceptors, and if any bacteria are introduced into the serum they unite with the free amboceptors in preference to uniting with those having complements attached. In this way the complements become diverted from the bacteria by the excess of amboceptors. It will also be recalled that while there are amboceptors in normal serum, these become increased when the animal from which the serum is obtained is injected with bacteria. The amboceptors formed by these injections are specific; that is to say, an animal injected with *B. choleræ suis* develops amboceptors in the serum which are capable of uniting with *B. choleræ suis* only. Reference to figure 4 (page 51) will serve to elucidate whatever may be obscure in the above explanation of the theory of complement diversion.

Bearing in mind that normal serum contains a relatively large amount of complement and a relatively small number of amboceptors, it is evident that by introducing normal serum into immune serum it is possible to increase the amount of complement without adding materially to the number amboceptors. In fact, by making a series of dilutions of immune serum and adding to each lot in the series a definite amount of normal serum, it is possible to obtain a set of dilutions containing various amounts of amboceptors and approximately the same amount of complement. Evidently if the immune serum is merely diluted and no normal serum is added, the complements present will be diluted in the same proportion as the amboceptors.

If the amount of complement is kept constant in the way above described, by the addition of a constant amount of normal serum, it is evident that in some of the dilutions of the immune serum the amboceptors will have enough or more than enough complements to combine with the amboceptors, and that in such cases the conditions necessary for bacteriolysis will be fulfilled—the amboceptors with the complements attached will unite with the bacteria. In other words, the undiluted immune serum, having an excess of amboceptors over complements, fails to show bacteriolysis, while the diluted immune serum with complements added, having no excess of amboceptors over complements, causes bacteriolysis.

Buxton[4] has raised the objection to the theory that it can account for those cases only in which the amount of complement is kept constant in the dilutions of immune serum, while the amboceptors alone are diminished. He makes the point that in experiments such as are recorded in the present paper, in which amboceptors and complements are diluted to an equal extent, the theory of complement diversion is inapplicable; and since the process is probably the same in both cases, both where the complement is kept to a constant amount by heating the serum and adding a definite quantity of normal serum on the one hand and where the serum is not heated and has no normal serum added on the other hand, the theory as a whole is not tenable. Valid as this objection appears from Buxton's presentation, it appears on further consideration as scarcely to be insuperable, for the theory seems to afford a satisfactory explanation in every case where the amboceptors are in excess of the complements, and, granting a certain proportion between these in the undiluted serum, it is not necessary for the complement to be present in an equal amount in the undiluted serum and in the dilutions for the occurrence of the diversion of the complement to take place. All that the theory would seem to demand is that the undiluted serum contain an insufficient amount of complement to satisfy the amboceptors which are free and those which are attached to the bacteria. Bacteriolysis will take place just in proportion to the paucity of the free amboceptors, for, according to the theory, those bacteria which are not coupled to free amboceptors are liable to bacteriolysis by becoming attached to the amboceptor-complement combinations.

In figure 4 it is attempted to show that complement diversion may well account for those cases in which the complement is diluted in the same proportion as the amboceptors. This point could be more strikingly shown if a larger number of amboceptors and complements could be taken, and a much greater relative difference could be made in the diagram between the number of amboceptors and of complements, but for obvious reasons it is impracticable to do this. Thus, if it were feasible to show 100 amboceptors and 100,000 amboceptor-complement combinations in 1 c. c. of undiluted serum, it could be demonstrated that 100 bacteria introduced would all escape bacteriolysis in this serum, only 10 bacteria would escape in 1 c. c. of a dilution of 1 to 10 of the same serum, only 1 bacterium would escape in 1 c. c. of a dilution of 1 to 100, and none would escape in 1 c. c. of a dilution of 1 to 1,000. In other words, theoretically in serum in which the above conditions prevailed, taking 1 c. c. in every case and introducing 100 bacteria, there would be no bacteriolysis in the undiluted serum, but complete bacteriolysis in the same serum in the 1 to 1,000 dilution.

In figure 4 the amboceptors are represented by the parts marked *a*, the complements by the parts marked *k*, and the bacteria by the parts marked *b*. No. 1 is intended to represent the undiluted serum, and all except two of the amboceptors are shown as having diverted a corresponding number of complements, while the two amboceptors, which are not united to complements, and which represent the excess of these bodies over complements, are united to a corresponding number of bacteria. Evidently in such a case there would be no bacteriolysis, since all the complements have been diverted from the bacteria. In No. 2 half the amount of the serum, as in the first case—

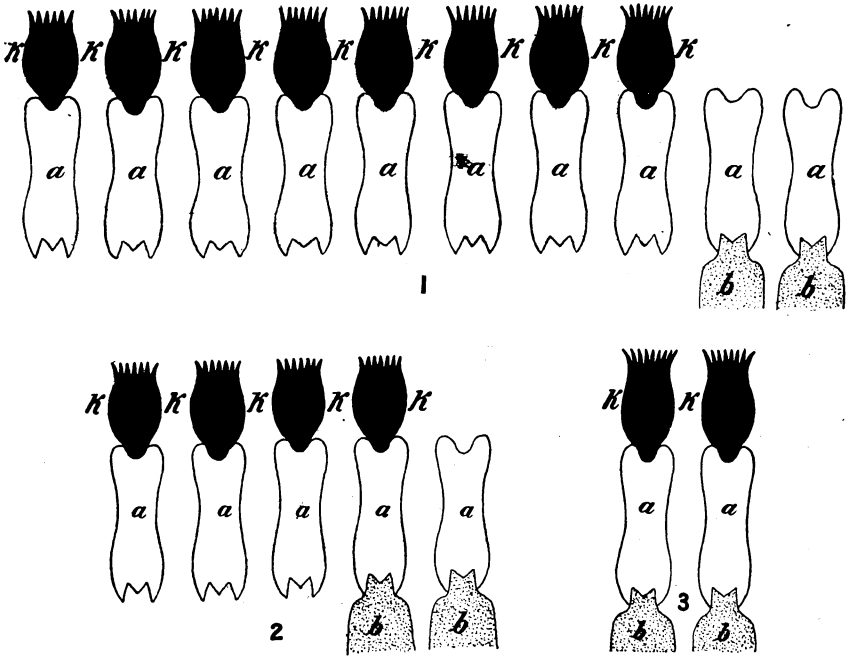

FIG. 4.—Diversion of complement in immune serum, not heated, and without the addition of foreign complement.

that is to say, 1 c. c. of a 1 to 1 dilution—is represented, with the same number of bacteria added as in the undiluted serum represented by No. 1. Under the conditions represented by No. 2 it is evident that one-half of the bacteria would be killed. In No. 3 is represented 1 c. c. of a dilution in which 0.25 c. c. of the original serum is contained. In this case all the bacteria would be killed, since there are no longer any free amboceptors to unite with the bacteria, or, at any rate, there would not be enough of the amboceptors to divert the complement in all of the four portions into which it is assumed the serum is divided. If the amboceptors are to be regarded as chemical substances in solution, then there would not be enough in any one of the four portions to cause diversion of the complement. If, on the other hand, the

amboceptors are bodies in the strict sense, then it is evident that two of the four portions would show bacteriolysis of one-half of the bacteria, while the other two would show complete bacteriolysis, assuming uniform distribution of the amboceptors. In either case the diluted serum would be more powerfully bactericidal than the undiluted serum. It will be observed that the complement is not kept at a constant amount in the dilutions represented by the diagrams, but that it is diluted simultaneously and to a like degree with the amboceptors.

From what has been said above it would appear that diversion of complement seems a reasonable explanation of the phenomena which are observed in unheated unreactivated serum, and it therefore seems to overcome Buxton's objection to the application of the theory to the phenomena observed under such conditions.

It is aside from the purpose of the present article to try to meet in general objections to the theory of complement diversion, or to discuss this theory apart from whatever bearing it may have upon the present experiments; but incidentally the objection raised by Buxton of bringing in accord with the theory of complement diversion the fact that Asiatic-cholera spirilla are killed about as readily by typhoid and paratyphoid immune serum as by normal serum may perhaps be overcome by consideration of the fact that the injection of an animal with any one kind of bacterium probably has no effect upon the amboceptor-complement combinations for any other kinds of bacteria which are present in the serum of the animal undergoing inoculation. The effect is probably very sharply specific, even going so far in some cases as to affect different strains of the same organism, as is shown by the results of some of the experiments in the present paper. It seems not at all inconceivable, at least, that an animal injected with any given kind of bacterium might well be deprived of the power of killing the kind of bacterium with which it was injected, owing to the excessive production of specific amboceptors, without interfering with the amboceptor-complement combinations for any other bacteria which may be originally present.

In view of the very specific reaction which takes place on injecting an animal with bacterial cultures, there would really seem to be no good reason why immune serum should act differently from normal serum for any but the homologous organism. The amboceptors which are newly formed in consequence of the injections could not reasonably be supposed to have the power of depriving the amboceptors originally present of the complements to which they are attached. If it is true, as assumed, that in normal serum there are amboceptor-complement combinations which will destroy *B. choleræ suis*, and in the same serum amboceptor-complement combinations which destroy *B. typhosus*, the injection of the animal with cultures of *B. choleræ suis* would evidently

increase the amboceptors for this organism, and these newly formed amboceptors would have the power of uniting with any free complement, but not with any complement already united to the *B. typhosus* amboceptors. So that while the newly formed amboceptors would and probably do find free complements with which to unite, they could hardly be expected to tear loose the bonds between the original amboceptors and complements.

As has been pointed out by Neisser and Wechsberg in their experiments, the increase of bacteriolysis usually observed on the dilution of immune serum does not appear to be explicable by the conception of Bordet that the immune body—the amboceptor—is merely a sensitizing agent. For if the amboceptor were in reality merely a "substance sensibilisatrice" it is not likely that immune serum would be more active when dilute than when concentrated. At least it would be only reasonable to presume that a reagent possessing the properties ascribed to the sensibilisatrice by Bordet and his followers would lose rather than gain in power upon dilution, and that a given amount of the undiluted sensibilisatrice would be more potent than the same amount of the diluted substance, as is the case with other reagents. On the other hand, Ehrlich's conception of the amboceptor affords a satisfactory explanation of the behavior of immune serum upon dilution, as has been shown in the above discussion of the theory of complement diversion.

The results obtained in the present investigation in which the hog serum appeared to increase in potency upon dilution seem best explained by the theory of complement diversion. But in these experiments, as in all others of like nature, it would seem possible that the process of bacteriolysis or the failure of bacteriolysis may at least be complicated by the formation of antibacteriolysins on the injection of the hogs or by the presence of antilysins normally present in the serum. Opinions are divided upon the question as to whether there are such antilysins present in normal or in immune serum. But if there should be such antibodies present, it is reasonable to suppose that these would be weakened in power on diluting the serum, and that consequently such serum would be more potent when diluted than when undiluted, unless the lysins and antilysins should be diluted in like proportions.

There seems to be no difference of opinion in regard to the development of qualities antagonistic to bacteriolysis in serum under certain circumstances, but the matter in dispute is whether this antagonistic property is due to special antilysins or whether it is due to the presence of nonspecific, normal amboceptors or of superabundance of immune amboceptors which absorb the complement, and in this way prevent bacteriolysis.

Pfeiffer and Friedberger,[16] who were the first to detect this antago-

nistic action on the part of normal serum, hold that under certain circumstances specific antibodies are formed, which combine with the complement. In other words, they regard the antilysins as anticomplements. Their experiments consist of triturating normal serum with bacteria and centrifugalizing. Under such circumstances the supernatant serum acquires the property of neutralizing the bacteriolytic power of immune serum. Thus normal serum, which in itself exerts no antibacteriolytic power, becomes antibacteriolytic for cholera immune serum when it is triturated with cholera bacilli and centrifugalized or filtered through a bacteria-proof filter.

Gay[10] attributes antilytic properties developed in this way to the formation of specific precipitines. He finds that only when there is a specific precipitation is there any antilytic property shown by hemolytic sera, and since hemolysis and bacteriolysis are probably identical processes, the same statement would apply to the latter as well as to the former.

Gay's view has been called into question by Sachs,[18] who attributes the antilytic properties of serum to normal, nonspecific amboceptors which he regards as acting as neutralizers of the complement. The only difference between the explanation offered by Pfeiffer and Friedberger on the one hand and Sachs on the other is that while the former look upon the antilysins formed in their experiments as newly formed bodies derived in part from the bacteria, Sachs contends that the antagonistic bodies are present in the serum from the start. He states, at least, that this is the only point in which he takes issue with Pfeiffer and Friedberger on the matter of the formation of antilysins for hemolytic serum.

Lipstein,[11] on comparing strongly agglutinating immune sera, found that while both the specimens examined possessed equal agglutinating power only one of them showed the phenomenon of complement diversion—that is to say, antibacteriolytic properties. He was also unable to discover any constituent of the normal serum which showed diversion of complement, but on the contrary he found this phenomenon to take place in immune serum only. In other words, the antagonistic action of serum to bacteriolysis he attributes solely to the diversion of complement by the specific, immune amboceptors.

In spite of this conflict of authority, it would seem at least that the Neisser-Wechsberg phenomenon permits of easiest explanation by the theory of complement diversion. For if the failure of the undiluted immune serum to produce bacteriolysis were due to the presence of antilysins, it would be less potent upon diluting the serum, it is true, and consequently exert less power upon the bacteriolysins when diluted than when undiluted, but the relative effect would remain the same. It is nevertheless probable that if bacteriolysins are present in immune serum they complicate the process.

While the theory of complement diversion seems to have been used by all investigators to explain the bacteriolytic phenomena of immune sera only, it would not seem inapplicable to those which take place in normal serum as well. The theory seems at least to explain the behavior of the normal serum of hogs upon dilution in the present investigation. Even in those cases where the normal serum showed diminution of potency on dilution, the diminution was not in direct proportion to the dilution, as has been previously noted; but on the contrary the diluted normal serum acts more powerfully, relatively, than the undiluted normal serum. This relatively greater potency of the diluted normal serum over the undiluted may be accounted for by the theory of complement diversion, if it is assumed that there is an excess of amboceptors over complements in the normal serum of hogs to start with, as certain considerations will show.

Difficulties in the way of the acceptance of the explanation just offered are of course very obvious. In the first place, normal serum generally has been found to contain an excess of complement over amboceptors; in fact, in adding normal serum to heated immune serum, this is done for the purpose of increasing the amount of complement without adding to the amboceptors. In heating immune serum it is the complement which is rendered for the time inactive, as it is called, and the addition of fresh serum reactivates the immune serum by means of the new complement contained. The addition of fresh serum to the heated typhoid immune serum of a rabbit reactivates the immune serum. On account of the large amount, or of the great number, of complements contained in the serum from the blood of a normal horse this can be used to reactivate several different kinds of immune sera. Then again, as stated above, in the process of immunization, the newly formed amboceptors probably find free complements with which to unite in the serum of the animal undergoing injection.

While the force of these and of possibly other objections can not be denied, it would seem evident from the experiments herein reported that the only difference between the behavior of immune serum on the one hand and of normal serum on the other, upon dilution, was a difference of degree and not of kind. If this is true, the theory of complement diversion must be applicable to both or to neither.

Evidently there are only three possibilities in regard to the relative number of amboceptors and of complements in any given serum—either there is just the proper proportion of amboceptors to complements or there is an excess of the one or of the other of these. If the amboceptors and complements are present in just the proper proportion, then there would be a simple relation between the undiluted and the diluted serum. The serum would become relatively and actually less and less potent in exact ratio to the extent of the dilution. If a given

number of bacteria were killed by a certain amount of the undiluted serum, a certain fraction of this number would be destroyed by the same fraction of the serum. If the complement is in excess, evidently the same rule would hold; the free complements would neither help nor hinder the process. But if there were an excess of amboceptors, a part of the bacteria corresponding to the amount of this excess would escape bacteriolysis, according to the theory of complement diversion. On dilution, however, there would be relatively more bacteria killed where there are free amboceptors present in the undiluted serum, as already explained in the description of the Neisser-Wechsberg theory.

Since none of the experiments in the present investigation have been made to determine whether the serum from normal hog's blood contains free amboceptors, and no experiments of the kind with hog's blood seem to have been so far reported, the matter must for the present be left undecided. But the assumption of such a condition in the normal serum would seem necessary to account for the results obtained.

A specific example taken from one of the experiments already described may serve to show the application of the theory of complement diversion to the phenomena observed on diluting normal hog sera in so far as the effect of dilution upon the bacteriolytic potency is concerned. Into the undiluted serum from the blood of hog 1859, and into each of the various dilutions, there were introduced before injection of the animal 3,760 bacilli of the Crawford strain per 1 c. c. of the serum. The number of bacilli surviving after twenty-four hours in the undiluted serum was 80 per 1 c. c. There are two conditions which may be assumed to exist in the serum, either of which would account for the escape of the 80 bacilli, disregarding for the present the fact elsewhere discussed that there is a multiplication of the bacteria surviving bacteriolysis and assuming that the 80 bacteria in the present case represent merely those bacteria which escaped bacteriolysis. Either there were not enough amboceptor-complements for all the bacteria introduced—in other words, there were 80 short of these—or, if there were enough or more than enough amboceptor-complements, there were also 80 free amboceptors. Either of these two conditions would account for the escape of the 80 bacteria in the experiment. A simple calculation from the result obtained on diluting the serum will show which of these conditions is most likely to have prevailed. If there had been a paucity of amboceptor-complements—if there had been, in other words, just 3,680 of these instead of over 3,760, which was the number of bacteria added—then a dilution of 1 part of the serum to 9 parts of salt solution should have killed only 368 bacilli per 1 c. c. when these were introduced; whereas, as a matter of fact, this dilution killed 2,600 per 1 c. c. There would appear, therefore, no ground

for assuming the failure of bacteriolysis in the immune serum to have been due to a paucity of amboceptor-complements in the undiluted serum.

Suppose, now, that the other condition mentioned above existed in the serum, namely, that there was an excess of amboceptors over and above the amboceptor-complements, and that there were more amboceptor-complements than the bacteria introduced. A simple calculation will show that such a supposition will readily account for the results obtained in the diluted serum as compared with the results in the undiluted. If there were in the undiluted serum 26,000 amboceptor-complements and 80 free amboceptors per 1 c. c. then on dilution of 1 to 10 there would evidently be 2,600 amboceptor-complements and 8 free amboceptors per 1 c. c. of the diluted serum. Consequently 8 bacteria would escape bacteriolysis on account of being attached to the free amboceptors, but 2,600 would be destroyed.

The above calculations are, of course, not intended to indicate precisely the processes involved, for these are probably complicated by the multiplication of the surviving bacteria, as mentioned above. Still, even if such a complication is admitted, the probabilities in favor of the condition last named become even greater, as a moment's consideration will show.

A further complication may also arise from the possibility, however remote, that the amboceptors and complements are not uniformly distributed in the serum—that while they may perhaps usually be so distributed, they may be found more abundantly in one part of the serum than in another.

SUMMARY OF THE CONDITIONS AFFECTING BACTERIOLYSIS.

The conditions under which bacteriolysis would take place, and those under which no bacteriolysis would take place in any given serum, may be summarized as follows:

1. Complete bacteriolysis could take place only where there were no free amboceptors and where there were at the same time a number of amboceptor-complements equal to or greater than the number of bacteria introduced.

On dilution in a serum of this kind there would be a loss of bacteriolytic power in proportion to the degree of dilution if the amboceptor-complements were exactly equal in number to the bacteria introduced. If there were more amboceptor-complements originally in the undiluted serum than the bacteria introduced, then on dilution there would be relatively more bacteria destroyed. If the excess of amboceptor-complements is large enough, there could of course be enough present in the diluted serum to kill as many bacteria as were killed by the serum before dilution.

2. Partial bacteriolysis would follow when there were fewer amboceptors present in the serum than the bacteria introduced and when at the same time there were any amboceptor-complements present.

The extent of bacteriolysis upon dilution would depend upon the number of amboceptor-complements present originally.

3. No bacteriolysis could take place if the free amboceptors were equal in number to the bacteria introduced, or if they were in excess of this number, either in the undiluted or the diluted serum.

THE THEORY OF COMPLEMENT DIVERSION.

In spite of the serious difficulties alluded to it would nevertheless appear that the theory of complement diversion gives an insight into the process of bacteriolysis occuring in normal serum in so far as this is affected by dilution—in those cases at least where the experiments show that the serum is actually or relatively more potent upon dilution than when undiluted—irrespective of the nature of the serum, whether this is normal or immune. But this would leave the results obtained in certain other experiments still unexplained. It will be recalled that in certain experiments the serum was found to be bactericidal when undiluted, that it lost in bactericidal potency on dilution, but that it gained in potency upon further dilution. It is these results which would be left unexplained by the theory of complement diversion as thus far discussed. One example of this behavior among others is to be seen in Table XXVI, in the serum of hog 1798, ten days after the inoculation of the animal with the F. 26 strain of *B. choleræ suis*. In one of the tests there were introduced into the undiluted serum and into the various dilutions in round numbers 800 Crawford bacilli. The result after twenty-four hours was that all the bacilli were destroyed in the undiluted serum, while in the dilutions of 1 to 50 and 1 to 500 there was an increase of about thirty-three fold and one hundredfold, respectively. But in the dilution of 1 to 5,000 there were only 140 bacilli remaining. In other words, the undiluted serum showed strongly bactericidal power, and the dilution of 1 to 5,000 also showed considerable potency, while the dilutions between these two showed at most but feeble power. If this result is analyzed, it will be seen that the serum appeared to act like normal serum at the start when undiluted and in the first dilutions, but that on further dilution it appeared to act like immune serum. This suggests that possibly the normal amboceptor-complements persisting in the serum may have been more potent than the immune amboceptors formed in consequence of the injection, that the first effect of dilution was to reduce the potency of the normal amboceptor-complements, and that further dilution enabled the immune amboceptors to produce the phenomenon of complement diversion. This would be quite in accord with certain views expressed elsewhere in this paper to the effect that

the injections of cultures or their products while they cause the formation of new amboceptors leave the normal amboceptor-complements unaffected.

In the above discussion it is assumed that the two bodies concerned—the amboceptor and the complement of Ehrlich, the sensibilisatrice and the alexin of Bordet—are capable of uniting and do actually unite independently of the presence of bacteria or of other cells. But Bordet[3] has recently published a series of investigations tending to show that the experiments of Ehrlich and Sachs[5], which constitute the chief evidence in favor of this view, are capable of quite a different interpretation from this, and that this interpretation is in fact not justifiable from the results of the experiments which consisted in the demonstration of the fact, not denied by Bordet, that ox serum will produce cytolysis only when the serum has in it amboceptors and complements simultaneously. It is not possible, as in some other cases, to produce cytolysis by sensitizing cells with ox amboceptors—that is, with heated ox serum—and, after washing these sensitized cells, adding complement—that is, fresh serum. Cytolysis with ox serum takes place only when the heated ox serum and some unheated fresh serum (horse serum was the kind used in the experiments) are employed at the same time. This is interpreted by Ehrlich and Sachs as showing that while the free amboceptors present in the ox serum will not unite with the cells they will and do so unite when they are previously attached to complements. But Bordet's results appear to show quite plainly that in this case the horse serum which was used as complement produces cytolysis quite independently of the ox serum, and that while cytolysis takes place more promptly when heated ox serum—ox amboceptors—are added, the ox serum is not necessary. Bordet therefore regards the experiments as showing that the heated ox serum acted merely as an accelerator of cytolysis.

Bordet summarizes his conclusions as follows:

> We see but one rational explanation of the peculiar action of ox serum—that there exists in the serum a peculiar substance capable of resisting heat of 56° C. and which remains unaltered for many months in this heated serum. The substance is probably of an albuminous or colloid character, and does not adhere to the normal corpuscles, but is precipitated upon the corpuscles which are previously charged with sensibilisatrice and alexin. We believe that it is a veritable process of glueing of absorption depending upon molecular adhesion. * * * In conformity with the statements of Ehrlich and Sachs, experiments show that the corpuscles of guinea pigs become hemolyzed in a mixture of fresh horse serum and of ox serum, the latter having been heated to 56° C., while they resist hemolysis if they are first subjected to the action of the heated ox serum and have the horse serum added subsequently. But the interpretation offered by Ehrlich and Sachs, according to which the sensibilisatrice furnished by the ox serum does not unite with the corpuscles unless it (the sensibilisatrice) is previously connected with alexin derived from the horse serum is not correct. In the first place, the sensibilisatrice, which plays a preponderating and most essential rôle, is not contained in the ox

serum at all, but is furnished by the horse serum. Consequently these sensibilisatrices behave like all of their congeners, in the sense that they do not require the presence of the alexin before they are capable of uniting with the corpuscles.

Finally, this interpretation leaves completely in the dark the very special peculiarities of the cases of hemolysis in question.

The peculiarity of ox serum consists in the presence of a certain element which resists 56° C., and also resists standing, and is of the nature of a colloid, doubtless albuminoid, and which, furthermore, is absorbed by corpuscles which have become charged with sensibilisatrice and alexin, but which remains free in the presence of normal corpuscles or of corpuscles merely sensitized—i. e., corpuscles treated with heated serum alone. The absorption of this colloid by corpuscles which have been treated with both sensibilisatrice and alexin has the effect of energetically agglutinating them and of rendering them more susceptible to hemolysis except under certain circumstances. * * * The absorption of the sensitized and alexinized corpuscles is very likely due to molecular adhesion, the preliminary treatment having modified the corpuscles in so far as their adhesive properties are concerned. Under these conditions the absorption may take place independently of the species of animal from which the corpuscles are obtained; it may even take place with the corpuscles of the same animal which furnishes the colloid, as in the case of ox serum.

Now, if the contention of Bordet as set forth above is correct, and if the two bodies concerned in cytolysis do not unite, then of course the theory of complement diversion must fall, since it requires as the first condition the union of amboceptors with complements. Nevertheless, there appears to be as yet no other explanation of the phenomena observed on diluting immune serum, and, as an attempt has been made to show in the present paper, there seems to be no other explanation of the behavior of normal serum on dilution.

CONCLUSIONS.

In summarizing the results of the present investigations it is seen—

1. That the bactericidal potency of the serum from the same hog varies from time to time.

2. That the serum from one and the same drawing differs in potency for different strains of *B. choleræ suis.*

3. That while the effect of standing is to weaken the potency, this effect is more marked in some specimens of serum than in others under the same conditions.

4. That the bactericidal power of the serum from the venous blood of hogs is not always more potent than that from the arterial blood.

5. That heating the serum at about 54° C. for thirty minutes inactivates the serum.

6. That the Neisser-Wechsberg phenomenon is sometimes seen in and sometimes missed from the blood of hogs injected with cultures of *B. choleræ suis.*

7. That the theory of complement diversion may not only account for the behavior of immune serum, but also for that of normal serum on dilution, or, at least, that the theory, if applicable in the one case, is equally so in the other.

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